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ESF—low budget but higher profile

A EUROPEAN SCIENCE FOUNDATION (ESF) moves closer to reality with the recent meeting in Stockholm of representatives of almost all western European countries at which, after strong competition from Dublin and Leiden, Strasbourg was chosen as the site of the foundation's offices. The general outlines of the statutes were also approved and the next meeting of administrators will be in November at which time the constitution should have been drafted. Membership now seems to be resolving itself—only Portugal (an understandable and presumably temporary absence) was missing from western Europe, Finland sent an observer and Yugoslavia has had second thoughts and dropped out. "The door is open", one delegate said, "for eastern Europe to join, and it would be my hope that within a few years the membership would be truly pan-European". Within a year a President and a Secretary-General should be installed and a secretariat of about ten is anticipated in Strasbourg.

The ESF has had an unusual history and it bears a deceptive name. It was created out of a desire to pre-empt, rather than in obvious response to an immediate need. Administrators throughout Europe saw the growing danger, a few years ago, of a deadening hand from the European Economic Community (EEC) seizing science policy and attempting to run science from Brussels. This caused alarm for two reasons: nobody had much stomach for Brussels bureaucracy and international science policy making cannot stop at the frontiers of the Common Market. The ESF thus emerged to pre-empt the EEC—it is freely admitted that the driving force was fear that the community would otherwise do something of its own. The title is somewhat of a misnomer since, at least in English-speaking countries, ESF suggests a European version of the American National Science Foundation. This it is not. Science has its more continental meaning of learning (the British Academy and the Royal Society are both involved), and it is not planned that the ESF should hand out grants for research.

Is all of this a good thing? Scientists in general display little interest in supranational schemes, whether international or regional, until they can see concrete examples of benefit to themselves that can be obtained no other way. Multinational ventures in providing expensive facilities are obviously approved by many, but international associations to cover specialisations are more ambiguously viewed and the ESF will fall into this category. To the extent that they organise grand conferences, publish bulletins and attempt to impose standards they are often ineffectual and even disliked. Their lack of funds for the support of research writes them off for many for whom the only organisations worth respecting are fund-giving organisations. And yet they fulfil one purpose which alone justifies their existence—that of representation direct to

governments. An international union may mean nothing to a scientist in an affluent and enlightened country, but to one in less happy circumstances it may mean the moral support that leads to a government grant, permission to work abroad for a spell or even such mundanities as the price of an airline ticket.

It is to this mode of working that the ESF should try to restrict itself. If it is seen to be an inexpensive operation (with a budget remaining below the half-million dollar mark), which can act quickly as a lobby for scientists when rational intervention is needed and which can act as a matrix on to which multinational schemes can be assembled but off which they can rapidly be removed, then it deserves support. If it attempts to become more grandiose, and a hunting ground for the power brokers and bureaucrats of science, it must be stopped by scientists themselves taking action.

Between now and November the administrators have one important job to do—to increase scientists' awareness of the existence of the ESF. A press release about the selection of Strasbourg had yet to reach Britain, a week after the meeting ended. When Sir Brian Flowers (Flora Europea as one wag described him) announced the selection to a dinnerful of physicists the stirring of coffee cups continued unabated. There is no plan that the articles of the constitution should be open for any public discussion before November. This last is particularly unfortunate. Although the point is made that they will in the main be boring, they must inevitably contain a description of what the foundation can and cannot do. Too often scientists exert themselves with organisations only to find themselves up against administrative answers—"we have no charter to do this", "we would like to help but our constitution forbids" and so on. It would be good to think that here was one organisation which was understood by European scientists before it began.

Nobody yet knows what the ESF will do. It will do nothing at all unless scientists can be persuaded to see its value and to use it intelligently. This means a higher profile in the next few months.

100 years ago



THE President of the Institution of Civil Engineers and Mrs. Harrison held a reception on Tuesday evening in the western gallery of the International Exhibition, at which over two thousand guests were present. In addition to the picture galleries and rooms containing machinery in motion, the west quadrant was open, and in it were placed illustrations of recent scientific inventions specially lent for the evening. With the exception of Mr. Crook's experiments showing attraction and repulsion accompanying radiation, and Tisley and Spiller's compound pendulum apparatus, all were applications of scientific inventions to the wants of life, if wicked war may be included among our wants, for Sir W. Armstrong, and other firms, sent models of appliances for the hydraulic mounting of large guns, whereby they can be placed in position with ease.

From *Nature*, 10, 54 May 21, 1874.



The significance of the invisible millionaire

Peter J. Smith investigates Mr. Hughes's increasing interest in the ocean floor.

HOWARD HUGHES, invisible millionaire and founder of Trans World Airlines and much else besides, is a celebrity; his comings and goings, his doings and undoings, and his deeds and alleged misdeeds seem to be a source of fascination to many in the western world. But earth scientists in general, and oceanographers in particular, should be taking a much more serious interest in the activities of Mr Hughes, some of which are likely to have severe repercussions for everyone who values the freedom to pursue research on, in and under the world's oceans. That such interest is apparently nil, or at least remains unexpressed, must be counted as one of the most remarkable examples of scientific hypocrisy since the hearts of the physicists who had worked on the Manhattan Project bled over the annihilation of Hiroshima.

The problem arises because Mr Hughes has recently sent to sea the most advanced marine mining vessel in the world, the Glomar Explorer, designed and built in secrecy in Los Angeles by the firm of Global Marine Inc. And joining this ship off the coast of Nicaragua will be a large submersible barge constructed in equal secrecy by the Ocean Systems Division of Lockheed. Naturally, few details of these craft are available, although the submersible is known to be a 90 m x 30 m roofed structure which will be held in place beneath Glomar Explorer by docking legs and tubular stability controls 45-m long. A steel pipe passing through the ship's bottom will then be connected by divers to a dredgehead 15 m wide on the barge, whence a dredge pipe weighing about 4×10^6 kg will be lowered several thousand metres to the ocean floor. At the bottom of the dredge pipe will be equipment to skim the material off the surface of the ocean floor rather like a giant vacuum cleaner.

The object of this paraphernalia is,



Glomar Challenger: played a part in the discovery of minerals.

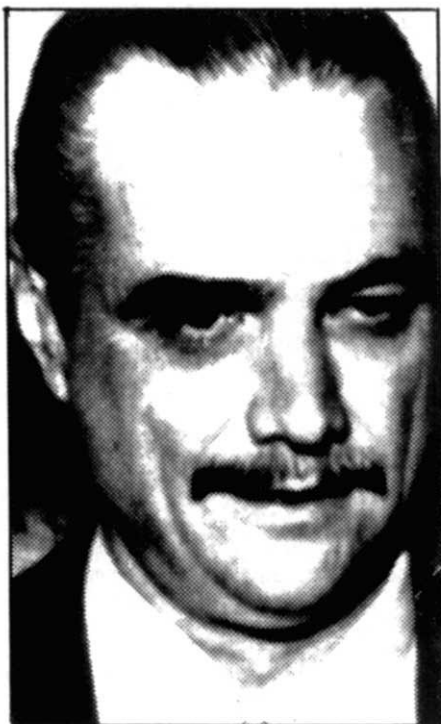
of course, to collect the copper, manganese, nickel and cobalt-rich nodules which lie on the floor of the Pacific; and although the Hughes equipment is still at the trial stage, this particular project is believed to be the most advanced of several undergoing development with similar aims. The rewards of success are likely to be high, which is presumably why Mr Hughes has been willing to invest so much hard cash in what seems at first sight to be such a risky venture. But the rewards of being first in the field will be what a rival of Mr Hughes has described as

“phenomenal”, for first-comers will have the joy of being able to undercut (some believe drastically) the high and ever-rising world prices for minerals.

It must be recognised, however, that any success that Mr Hughes might have in mining the ocean floor has important implications for anyone whose interest in the oceans is purely scientific. For one thing, it is already clear that Mr Hughes must have profited greatly from Global Marine's experience in the building, equipping and operating of the Glomar Challenger, the research vessel that has been used extensively by oceanographers to drill the oceanic crust in the search for evidence to support the new global tectonics. This is true not only of the technology involved but also insofar as the Glomar Challenger has played some part in the discovery of the minerals themselves. So one question which needs to be asked is whether it is right that an individual or company with purely commercial interests should benefit so directly and obviously from a project financed from public funds. The issue is an important one, even though it may not be possible in the end to reach a consensus on the right policy to adopt.

To the extent that the principal participants are American, it may also be thought to be an issue with political ramifications largely internal to the United States. But it is not quite as simple as that. The fact is that much of the mineral wealth of the ocean floor that is likely to be mined lies below what, for want of a better phrase, are called international waters; and some companies with interests in this field have been reported as saying that they will move into such waters whatever

Hughes: first in the field



anyone else may think, say or do. One of the practical advantages of so doing, of course, is that profits will be enhanced by the lack of mining taxes payable. But more important, any such move into international waters by a private company, or even by a single nation for that matter, would be contrary to a United Nations resolution declaring the riches of the ocean a common heritage to be shared by all mankind. If this resolution is contravened, and if the forthcoming Law of the Sea Conference fails to agree on a policy to control ocean exploration for the common good (or even if the conference agrees a policy which is later contravened), then as Lord Ritchie-Calder has pointed out, "it isn't hard to see armed conflicts between nations as a result". Moreover, Ritchie-Calder notes that "Hughes may have found a new place to hide—the bottom of the sea—but he won't long be there alone. These industrial privateers are going in for a system much like opencast [strip] mining. Some will do their primary refining at sea, dumping acid and alkali

line wastes and tailings overboard. Nobody knows what effect this will have on ocean ecology".

None of this may come to pass, of course; but it affects earth scientists and oceanographers nonetheless, simply because it is possible. And this is where

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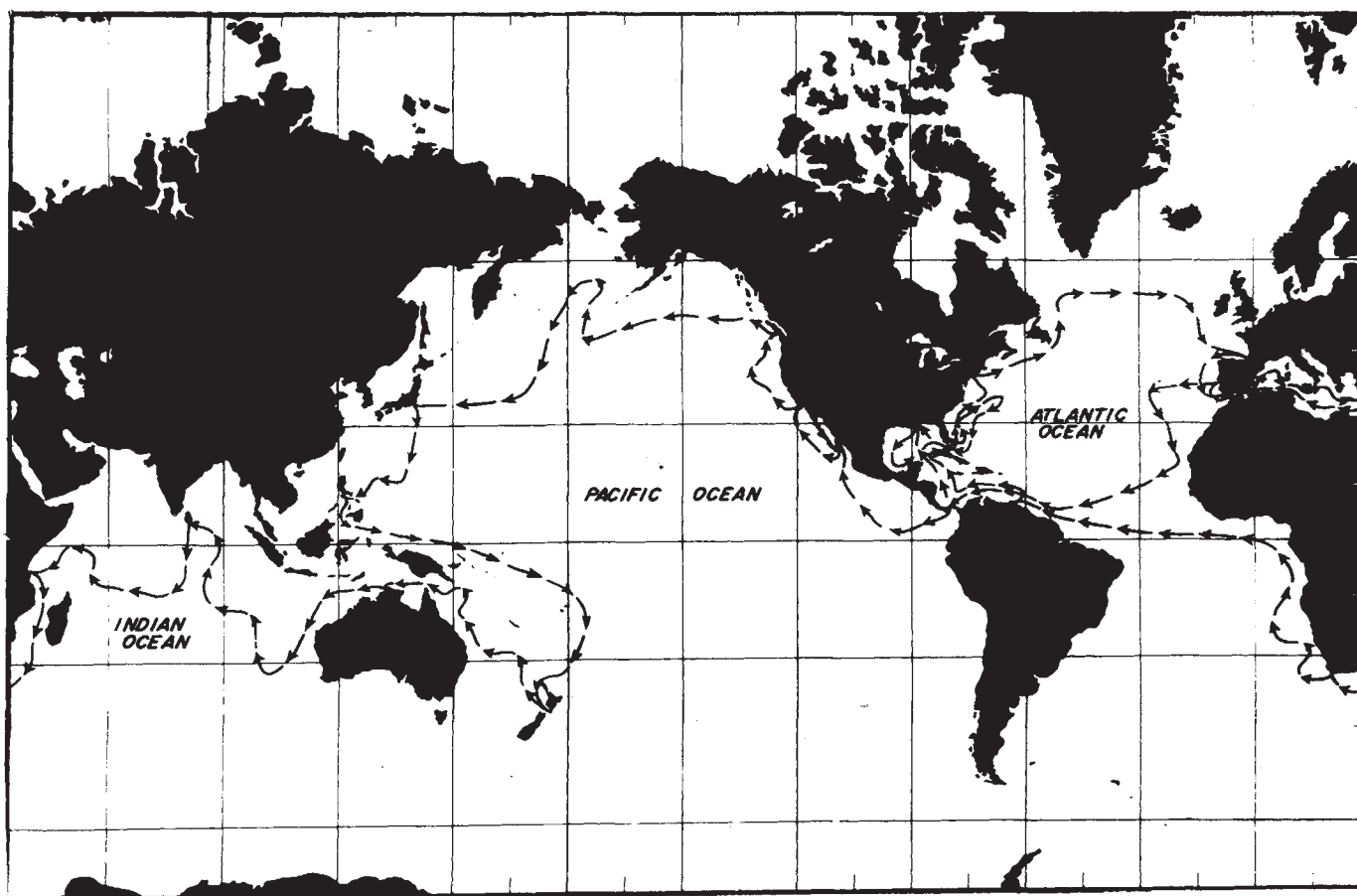
the hypocrisy comes in. During the past year or so several oceanographers have gone out of their way to deplore publicly the threat to basic research from ever expanding national claims to sovereignty over what are now regarded as international waters. The threat is real, but those writers who publicise it disingenuously avoid saying anything

about why it exists. One of the reasons is precisely the fear and suspicion of being exploited by more powerful nations—fear and suspicion which are fed by the activities of Howard Hughes and those with similar aims. Oceanographers, and oceanographers in the United States in particular, really should not underestimate the strength of feeling on this issue, not only in the so-called underdeveloped countries but also in those industrialised nations unable to afford the technology for undersea mining.

There is no doubt a real danger that certain countries will seek to prevent or restrict basic research work by outsiders within several hundred kilometres of their shores, and this may be a blow to science. But if this is to be avoided, these oceanographers who deplore the danger must be equally outspoken against those who feed the fear of exploitation; and the earth science community as a whole must make it clear where it stands on the "common good" resolution passed by the United Nations.

OCEAN drilling routes. For 30 months during the extension period of the Deep Sea Drilling Project, the Glomar Challenger followed the routes indicated below in three oceans and the Mediterranean Sea. The extension drilling was planned for 15 legs of duration two months each, starting in the Gulf of Mexico in February 1970. Other legs followed in the Atlantic Ocean, Mediterranean Sea, the Pacific Ocean,

Indian Ocean and ending again in the Atlantic Ocean. These routes followed closely areas expected to yield information concerning the interactions of the movements of continental and oceanic crustal areas. Scripps Institution of Oceanography was managing institution for the project under contract to the National Science Foundation. Builders of the vessel were Global Marine Inc., of Los Angeles.



international news

DURING the past couple of weeks, the Atomic Energy Commission (AEC) has been taking a savage public battering over its plans to develop the liquid metal fast breeder reactor (LMFBR) as the keystone to the entire nuclear power programme in the 1990s and beyond. Charges of biased statements, suppression of information, outright deception and other underhand dealings, which are not unusual in Washington these days, have been levelled at the AEC while it has been scrambling to meet a court-ordered deadline of June 14 to produce a final detailed analysis of the environmental, economic and social implications of the LMFBR programme.

Although the AEC is by now used to impassioned attacks by environmentalists and nuclear critics, many of whom are bent not just on stopping the LMFBR programme but also on halting the rest of the nuclear power industry, it may have a more difficult time in defending itself against a particularly devastating critique which was delivered early last week, for it came not from avowed nuclear critics but from another agency in the federal government—the Environmental Protection Agency (EPA).

It is not difficult to see why the LMFBR programme has raised passions in the United States, for in the words of Barry Commoner, the outspoken environmentalist and leader of an organisation called the Scientists Institute for Public Information (SIPI), the country is now "at a crossroads" in its energy policies. A decision to push ahead rapidly with the development of the LMFBR would lock the United States into a nuclear-dominated energy supply programme if for no other reason than the huge cost that the LMFBR would entail. On the other hand, if nuclear critics can nip the LMFBR programme in the bud, they argue that the federal government would be forced to pay more attention to the development of renewable energy resources and energy conservation. The LMFBR has thus become a rallying point for a powerful coalition of environmentalist and scientific groups.

The debate is centred on a landmark decision of the federal appeals court which last year forced the AEC to obey the dictates of the National Environmental Policy Act (NEPA) by producing a broad and detailed analysis of the environmental impact of the commercial

Concerted criticism of breeder reactors

Colin Norman, Washington

application of several hundred LMFBRs. The decision forced the AEC to examine the advantages and disadvantages of the entire LMFBR programme in relation to other methods of producing energy, and the impact statement should therefore form the basis for a public examination of the United States energy policies.

The AEC produced a draft of the NEPA impact statement on March 14 and it is legally empowered to put the statement into final form by June 14. It is the draft statement which is the basis of the AEC's public mauling. The Natural Resources Defense Council (NRDC), a public interest law group, has charged that the draft statement is "a deliberate attempt to mislead the public" and has demanded that it be withdrawn and rewritten. Similarly, the SIPI has castigated the AEC for producing an impact statement which is "so grossly inadequate as to prevent rather than enhance public discussion of the AEC decision to proceed with the programme". And finally, although using rather more restrained language, the EPA has given the AEC's draft statement its lowest possible rating and said that it "does not adequately detail the potential environmental impact" of the programme. In fact, the EPA said that the document was so deficient that it does not provide enough information to allow anybody "to reach the conclusion that the present LMFBR programme is preferable to other energy options".

The EPA's critique has placed the AEC in an unenviable position, for it has hit a number of the most sensitive points in the AEC's justification for the LMFBR programme. If the AEC does alter its statement accordingly, a number of its chief arguments could well disappear, whereas if it ignores the EPA's comments it will lay itself wide open to a court suit by environmentalist groups.

One of the AEC's chief justifications for the LMFBR programme is that breeder reactors are much more efficient than conventional light water reactors

(LWRs) in their use of scarce uranium resources. The LMFBR can take uranium-238, which is unsuitable for use in LWRs, and convert it to plutonium which can then be used to fuel both conventional and breeder reactors—it breeds fuel, in other words. Since the AEC predicts that the United States has only enough uranium left to fuel conventional reactors for another 25–50 years, this characteristic of the breeder reactor will be essential for stretching out uranium resources, and it will make the LMFBR the keystone of the nuclear power programme.

The AEC's draft statement estimated that if all goes well the breeder reactor will be introduced in 1987, that 400 of them will be operating by the year 2000 and producing 400,000 MW of electricity and that by the year 2020 LMFBRs will be generating 2.2 million MW. The commission argued that there is no other technology on the horizon which seems capable of supplying that much energy and that to abandon the LMFBR programme now could well plunge the United States into a severe energy crisis towards the end of the century.

The AEC paints this picture of what will happen if the LMFBR is not introduced: as uranium resources dwindle, the cost of mining uranium ore will increase, and this in turn will increase the cost of generating electricity by LWRs and drive up electricity prices. A central theme is that the later the breeder is introduced, the smaller will be the benefits it produces, and therefore a strong commitment must be made as early as possible to the LMFBR programme.

But the NRDC has pulled the AEC's cost-benefit analysis to pieces, suggesting that it is founded on dubious assumptions, unrealistic projections and misleading accounting procedures. And the EPA has backed up most of the NRDC's complaints, suggesting that if the cost-benefit analysis is corrected, it would "preclude acceptance at this time of the AEC contention that deferral of the present LMFBR programme would be intolerably costly". The critique goes on to suggest that there is no reason to rush into the LMFBR programme because "there is sufficient time to consider alternative uranium-saving nuclear technologies and study and resolve some of the issues of environmental risk".

Those statements and the accompany-

ing documentation in the EPA's 94-page critique of the impact statement are likely to provide environmentalist groups with powerful ammunition if they eventually go to court to get the LMFBR programme stopped and, according to one EPA source, if the AEC does amend its impact statement by incorporating some of EPA's criticisms, the economic justification for the breeder could be shot to pieces.

As for the question of plutonium toxicity, that is likely to provide a considerable headache for the AEC for a long time to come. The draft NEPA statement concludes that the entire breeder reactor programme is likely to cause only 2.2 deaths in the United States through radiation from power plants, fuel reprocessing plants and waste disposal facilities. This statement was made in spite of acknowledgments elsewhere in the AEC's document that there is a good deal of uncertainty about the health effects associated with plutonium.

One of the chief uncertainties is the question of potential damage to the lungs from tiny particles of plutonium which have been inhaled, a topic which has recently been thrust into the public spotlight by two nuclear experts working for the NRDC, Dr Arthur Tamplin and Dr Thomas Cochran. Even before the draft impact statement was published, Tamplin and Cochran launched a vigorous attack on the AEC's radiation standards, contending that they are based on an unrealistic assumption that radiation from inhaled plutonium particles is evenly distributed throughout the lungs. They argue that tissue immediately surrounding a particle of plutonium is getting a radiation dose of about 4,000 rems a year, but if the dose is averaged out over the entire lung, it amounts to only 0.003 rems per year. They therefore argue that the AEC should modify its regulations governing the release of plutonium into the atmosphere by a factor of 115,000.

Although the EPA critique takes up no position on the validity of Tamplin and Cochran's theory, it says that "a scientific basis for estimating health effects from plutonium particulates is not available at this time". The EPA therefore recommends that the AEC's final environmental impact statement should indicate what steps the commission would take if the Tamplin-Cochran thesis turns out to be correct, and it also suggests that "as a final alternative, the commission may want to examine what tradeoffs exist in delaying the implementation of a full-scale commercial LMFBR programme until the risks from particulate doses, as compared to those from a uniform lung dose, are on a more firm scientific basis so that meaningful public judgment on the risks and benefits of the programme

Commoner lays into AEC

ONE of the most stinging criticisms of the Atomic Energy Commission's draft environmental impact statement on the liquid metal fast breeder reactor (LMFBR) programme has come from Barry Commoner, an outspoken champion of the environmentalist movement. Commoner has charged in two press conferences that the Atomic Energy Commission (AEC) has been deliberately suppressing information on the potential of solar energy in order to make its case for the breeder reactor look good.

The accusation stung, for AEC chairman Dixie Lee Ray immediately put out a statement labelling the charge "totally false" and has steadfastly maintained that the commission is not trying to suppress any information relating to the LMFBR programme.

The nub of the dispute is this. Commoner, who is chairman of the Scientists' Institute for Public Information (SIPI) maintains that the AEC's draft impact statement fails to take into account a very optimistic report on the potential for solar power which was prepared for Dr Ray last year. The report, prepared by a panel of experts which assisted Ray in putting together her recommendations for a five-year energy research and development programme, concluded that solar power technologies could be developed to produce about 21% of the electrical energy needs of the United States by the end of this century.

The AEC's draft statement, how-

ever, said that "the outlook appears to be that solar energy has little potential as an economic, major source of electricity for several decades" and it went on to suggest that "the use of solar energy will not materially reduce the need for alternative electrical energy sources in the foreseeable future". The impact statement makes no reference at all to the panel's report.

The dispute about solar energy is central to the debate about the need for the LMFBR programme because, if the panel's projections prove to be correct, solar energy would be able to supply almost as much power as the breeder reactor by the year 2000 (see Table 1). And that would shoot to pieces the AEC's contention that the breeder reactor is desperately needed to avoid an energy crunch toward the end of the century. Commoner contended in his first press conference on the matter that "the AEC may be involved in another Washington coverup—this time an attempt to cover up the Sun".

According to the SIPI, which together with the Natural Resources Defense Council brought the suit which forced the AEC to produce the impact statement on the LMFBR programme, the statement's failure to consider solar energy as a viable alternative to the breeder programme is "a gross dereliction of the AEC's duty to the demands of NEPA [the National Environmental Policy Act] and the courts".

Table 1 Summary produced by SIPI of contributions to projected United States electrical demand (year 2000)

Energy source	Generating capacity (millions kw)	Percentage of projected demand
Solar technologies		
Photovoltaic	140	7
Solar thermal	40	2
Heating and cooling of buildings	35	2
Wind	170	9
Ocean thermal	Not available	Not available
Bioconversion	25	1
Total, solar technologies	410	21
Geothermal	80	4
Conservation of electricity	236 (minimum)	12 (minimum)
Grand total	765	37
LMFBR programme	435	23

is possible".

As for the safety of the LMFBR, the EPA critique of the draft impact statement says that the AEC has not been sufficiently qualitative in its assessment for the EPA examiners "to conclude what safety approaches will be taken in many important areas". It is suggested that the AEC should include in its final impact statement a comparison of the risks of the LMFBR and the LWRs.

In view of the serious deficiencies which have been unearthed in the AEC's draft impact statement, the EPA

has recommended that AEC lawyers should go to court to get an extension of the June 14 deadline for producing the final impact statement, while the SIPI and the NRDC have suggested the more drastic step of writing another draft statement and following that up with a final document.

Whether or not the AEC sticks to the present deadline, it can be certain that its final impact statement will be challenged in the courts if it fails to meet the objections raised by EPA or other critics

Alkali Inspectorate still at first base

Eleanor Lawrence

HER Majesty's Alkali and Clean Air Inspectorate comes under strong criticism in a recent report for its attitude to the problems of pollution control which, says the report, is now out of date and often ineffective. (*The Alkali Inspectorate*, Social Audit, 9 Poland Street, London W1V 3DG, £1).

The report, prepared by Maurice Fraenkel for Social Audit, criticises the inspectorate for not publishing the detailed data it collects and for general secrecy and non-cooperation towards members of the public and local authorities who make complaints against specific works. Also, says the report, it seems unwilling to put the maximum pressure on factories who do not comply even with its standards.

These specific criticisms stem from the way the inspectorate itself sees its role in controlling industrial pollution. In the words of the present Chief Alkali Inspector, Mr F. E. Ireland, the first Chief Inspector, Dr Angus Smith in 1863 "set the policy pattern of the inspectorate which has been followed ever since". This policy involved a sympathetic approach towards the manufacturers which included technical help towards solving their problems which was very welcome and did result in the early days of the inspectorate in a tremendous reduction of pollution. But, says the report, times have changed and this attitude may not be quite so effective nowadays and is in fact blocking the way to a more effective control of pollution.

"We like to help industry", Mr Ireland told the author of the report, "when we visit a works they are actually glad to see us." But from the outside, local authorities and members of the general public have made many complaints that the standards that the inspectorate itself lays down are often not being followed by the factories and that the inspectorate will allow works more than ample time to put their house in order even when this results in serious nuisance to local residents.

Local authorities which, through their own Public Health Inspectors, control pollution from all the factories and processes that are not registered under the Alkali Act, are one of the main critics of the Alkali Inspectorate. Some of the larger authorities have tried to break into the mutual admiration society obtaining between the inspectorate and the industries they monitor by suggesting that they should take over the inspectorate's duties, but this suggestion has been repulsed both by the inspectorate and by the industries themselves, who are

seemingly unwilling to leave the inspectorate's protection.

Many of the complaints about inefficiency on the part of the inspectorate can be traced to its staffing which is described in the report as totally inadequate. Thirty-five inspectors monitor the 2,000-odd works registered under the Alkali Act and in these circumstances even the inspectorate admits it is unable to carry out the job as well as it would like.

The report concludes that despite its proud record and the undoubted technical expertise and determination of its staff, the Alkali Inspectorate is particularly ill-suited to the role now expected of it. It suggests that the simplest solution to the problem might be to transfer the responsibility for control of registered works to the local authorities, which nowadays have the technical skills needed to deal with this sort of work.

If the Alkali Inspectorate is to continue as an enforcement agency, then it needs more staff and more frequent inspections; but most of all, says Mr Fraenkel, it must be made accountable and all pollution data other than genuine industrial secrets must be published in a readily available form. The inspectorate's defence of its policy of non-publication is that the public does not understand pollution data.

Azbel's visa

WE have just received news that Professor David Azbel, one of the three Jewish intellectuals who went on hunger strike "taking a step befitting political prisoners" earlier this year (*Nature*, **248**, 2-3 (1974)) has received a visa permitting him to leave the Soviet Union. No visas have as yet been granted to his fellow strikers, Vitalii Rubin and Vladimir Galatskii.

Happy birthday, Soviet style

Vera Rich

As readers will be aware by now, the 250th Anniversary Meeting of the Soviet Academy of Sciences has been postponed. A brief note in the *Pravda* of May 6, 1974 (which appeared on page 2, the page devoted to Party and political news) announced:

"The Academy of Sciences of the USSR with the approval of the Central Committee of the Communist Party of the Soviet Union has resolved to postpone the meeting devoted to the 250th anniversary of its foundation, from May, 1974, until a later date. During this time, celebratory (anniversary) meetings of the Academies of Science in the Union Republics will be

held with the participation of Soviet society. Meetings devoted to the anniversary of the Academy of Sciences of the USSR, and to problems of the better implementation of scientific achievements in practice, will also be held in scientific institutions and establishments. It is intended that scientists will speak at industrial enterprises, collective farms and state farms with the aim of popularising scientific knowledge among the workers. The Academy of Sciences will complete its preparations and the publication of fundamental scientific works in honour of the anniversary, and will organise thematic exhibitions under the slogan, 'The Achievements of Science are for the National Economy'."

In these brief words, the preparations and expectations for a grand international celebration are 'postponed'—a word which may be taken in its literal sense, but which under the Stalin bureaucracy was a frequent euphemism for 'scrapped'.

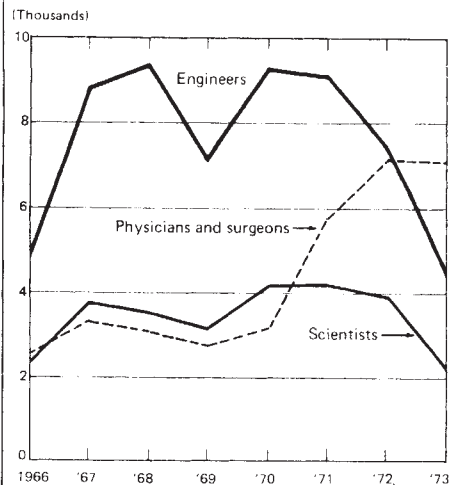
There is of course a certain suggestion of unpreparedness here—that although the resolutions to celebrate the anniversary were published last autumn, the Academy is still in a state of unreadiness. In spite of the Soviet predilection for the fulfilment of norms ahead of schedule, such lack of preparedness for conferences has been known to occur.

Even if there has been some tardiness in preparation, however, this is not the full story, particularly as the date of the 'foundation' of the Academy can be interpreted in more than one way. What it was intended to celebrate this year was the issuing of a decree by Peter I ("the Great") establishing the Academy, on January 28, 1724. Having begotten the Academy, however, Peter died and the inaugural meeting took place nearly two years later, on December 27, 1725, in the presence of Peter's widow and successor Catherine I. On at least one occasion, the 1725 date has been celebrated as the anniversary; in 1945 a 220th jubilee celebration was held and, although under war conditions a 1944 celebration would have been impossible, there was nothing in the official publications about the occasion to suggest that this was in any sense a postponement.

Looking closer at the announcement, however, it is not merely a postponement; it is a change of aim and intention. Instead of a grand international gathering (which has been 'postponed'), there are concrete plans for lectures on the grass roots of Soviet society and a stress on scientific utilitarianism.

But would it have been an international meeting at all? Would the leading scientists of the world really

Brain drain reduced to trickle



THE huge influx of scientists and engineers into the United States during the late 1960s, which became popularly known as the 'brain drain', has been sharply curtailed by restrictive immigration regulations imposed by the United States Department of Labor. At the same time, however, physicians and surgeons are flocking to the New World in record numbers.

According to statistics published by the National Science Foundation, some 6,600 scientists and engineers entered the United States between July 1972 and June 1973—the first year in which the full force of the new regulations was felt. This compares with an annual influx of from 10,300 to 13,300 between 1967 and 1972. As for physicians and surgeons, 7,100 were admitted to the United States last year, more than twice the number corresponding in the late 1960s.

Designed to protect the jobs of American citizens against competition from cheap foreign labour, the new immigration regulations specify that an alien can work in the United States

only if he has been offered a job which cannot be filled by a suitably qualified indigenous worker. Since there has been considerable unemployment among scientists and engineers stemming chiefly from cutbacks in expenditure on aerospace activities in the late 1960s, non-American citizens from those professions have a tough job getting round the regulations. By the same token, however, because there is a shortage of physicians and surgeons in the United States, qualified doctors are flooding into the country in increasing numbers.

The sharpest reduction in immigrant scientists and engineers between 1972 and 1973 was in the number of qualified Asians allowed to enter.

One sad irony is that scientists and engineers from India, for example, are being refused entry to the United States although there is massive unemployment among college graduates in India. At the same time, physicians and surgeons are flooding into the United States when they are badly needed in their home countries.

have gathered in Moscow to honour the Soviet Academy as a member of the international fraternity of science. With Academician Sakharov pilloried in the Soviet press as unworthy of the dignity of a Soviet scientist? With Jewish scientists who dare to apply for a visa for Israel submitted to all forms of harassment, from dismissal from their posts to petty and often puerile vexation, such as the ostentatious shadowing for no reason but to annoy? With an "opposition" seminar planned to be held in July in the Moscow apartment of one such dismissed scientist, Professor Aleksandr Voronel? With the British Royal College of Psychiatrists demanding in no uncertain terms last November an "impartial commission of enquiry by a broadly based group of psychiatrists of international repute, drawn from a number of countries", to investigate "the reports it has received about the alleged abuse of psychiatry in the management of individuals who take up a position of political dissent" in the Soviet Union?

In such circumstances, an international gathering might well prove too explosive a celebration. But the postponement announcement does not have the air of simply waiting for the tension to cool. The return to the grass roots, to the factories and farms, has the air of a total rethinking of how the event should be celebrated. If the outside world is critical of how the Soviet Union conducts its science, the Academy is willing to go out into the highways and byways and celebrate with the workers and peasants.

Start for UK on radiation

from our Solid State Physics
Correspondent

BRITAIN had its first meeting on radiation effects in electronic devices on April 24—a one-day event organised by the Institute of Physics (IOP) at Imperial College, London. The meeting is notable mainly because of the general issues it raised. In the United States there has been an annual three-day meeting on this topic since 1964, the proceedings of which run to 250 pages but ten years later, the United Kingdom has had a mere one-day meeting with no published proceedings. The organiser, Mr K. J. S. Cave of the IOP Electronics Committee, termed the lag "regrettable".

Why are the levels of interest so disparate in the two countries and is this apparent lack of interest within the United Kingdom justified? In answer to the first question, the main difference could derive from differences in the size and philosophy of defence and space programmes in the two countries, rather than from differing extents of use of radiation sources for more mundane purposes (such as nuclear reactors, processing irradiators, ion implantation, radiography or basic research). A fair proportion of the physical investigations has been done in the course of producing 'radiation-hardened' semiconductor devices for the United States Department of Defense and, to a much lesser extent, for

the National Aeronautics and Space Administration (NASA). The equivalent bodies in the United Kingdom government are not such expansive customers, although a few exploratory development contracts have been let by the Ministry of Defence. The difference here lies in the fact that United States strategic missiles must withstand nuclear attack, whereas the United Kingdom government has no such requirement. Space agencies in general seem to sponsor less work in this field in proportion to their need, partly because only a certain group of satellite trajectories intersect space radiation belts and perhaps the planners presume that they can avoid these—or fudge a solution.

As to the second question, the measure of justifiable interest for physicists should be the degree of understanding of physical processes which derives from the research. It is widely agreed that the physicist who studies the effect of radiation in an electronic device usually ends up by knowing more about that device than the man who designed it. Thus, we can say that the device physicist should gain benefit from such studies. Furthermore, it is common that investigators have to improve the general level of knowledge of radiation-induced defects in solids in order to formulate improvements in the 'radiation hardness' of a device. Thus, the physicist with an interest in the defect state of solids should find such work a suitable challenge. Similar arguments can be developed for ceramics, metals, plastics and other bulk materials and it can be said that the return in basic

knowledge is rather higher than is usual for such tactical research. On this reasoning, scientists in the United Kingdom may be missing a few tricks by not doing more work on radiation effects on their own behalf.

The planners may also have missed a trick here. Developing a radiation-tolerant material or device takes a very long time. In several advanced technological developments, radiation damage may often provide the limit to the usefulness of system: examples of such systems are fusion reactors and unmanned space vehicles. To obtain true effectiveness in research on radiation effects, the lead time may need to be well over ten years and

start long before engineering designs take shape. A surprising amount can be gained by research which prevents device designers from pursuing the goal of radiation resistance in the wrong way. The analogy with preventive medicine is, in fact, strong and probably again extends outside the field of intricate semiconductor devices, with which the meeting in question was concerned. Because there is not, as in the United States, a well developed 'radiation effects community', the need for both underlying and short term research in this speciality may not get pressed as strongly as it should in the United Kingdom—there is no one to give the whole picture to the top level technological

planners. In fact, it is notable how the 'reactor people', the 'device people', the 'dosimetricians' and other specialists, who have a lot to gain from each other's experience, very rarely join forces either in conference or Whitehall corridor to express a common interest in two basic processes with which they are all concerned, namely displacement damage and ionisation effects. A happy exception to this is the case of the ion implantation specialists, whose techniques are useful both to reactor and device designers and whose meetings embrace both fields. This suggests that it is time for a professional body to sprout a committee which does the job of bringing together specialists.

correspondence

Referees in print

SIR,—I read the recent letter by H. Fraenkel-Conrat [*Nature*, March 1] concerning anonymity of scientific reviewers with some interest.

I wonder if many readers are acquainted with the compromise system currently being used by the *Journal of Geophysical Research (Space Physics)*, and instituted by my predecessor, W. I. Axford. Each manuscript sent out for review is accompanied by a form stating that the journal acknowledges the assistance of reviewers by naming them at the end of the paper when it finally appears in print. The reviewers are asked to indicate on the form whether they agree to being acknowledged. If they agree, the paper appears with a footnote that states: "The Editor thanks A. Smith and B. Jones for their assistance in evaluating this paper". If one of them does not agree, the statement reads: "The Editor thanks A. Smith and another referee for their assistance in evaluating this paper". If both disagree, of course, no footnote appears, but many such papers are rejected in any case.

This rather simple device has worked very well since its inception; more than 90% of our reviewers do agree to this form of acknowledgment. It not only provides some small tangible acknowledgment of the reviewer, but it also apparently reduces the incidence of irresponsible reviews that are written solely for "the joy of releasing adrenaline with anonymous impunity", as Fraenkel-Conrat describes one such effort.

To my knowledge, no other journal has yet adopted the system in spite of

its demonstrated success and general acceptance among scientists in the field of space physics. It does carry with it certain corollaries that may not be acceptable to many editors—for instance, reviewers' reports must be transmitted to authors in a more or less unadulterated form, since it would hardly be fair to acknowledge a reviewer publicly for a report that had been translated and heavily doctored by the editor himself.

Yours faithfully,

GEORGE C. REID

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National Oceanic and Atmospheric
Administration,
Boulder, Colorado 80302*

Soviet scientists

SIR,—Professor Burhop states in *Nature*, April 12:

"... In Western society too much concern for problems of peaceful coexistence, disarmament and the abolition of nuclear weapons is regarded as at best naive and at worst subversive. In Soviet society these things are highly regarded."

It is reassuring to think that the crowds assembled in Moscow's Red Square on May 1 and November 3 are there to protest against the parade of intercontinental missiles with nuclear warheads that we see in the news photographs. Their protest is made impressive by the fact that it is expressed as applause, which makes it more orderly.

Professor Burhop also says that Soviet scientists who wish to emigrate to Israel bear a resemblance to Oswald Mosley in 1939. This lacks verisimilitude. The British Fascisti (the BBF of

Aldous Huxley's *Point Counterpoint*) did not try to leave, even though many people wished they would.

Yours faithfully,

THOMAS H. JUKES

*University of California,
Berkeley, California 94720*

Lead in petrol

SIR,—In the second paragraph of the article "Lead in the Environment" (*Nature*, April 5, 1974), the writer states that in 1972 about 90,000 tonnes of lead were added to petrol in the United Kingdom alone. No reference is made to the source of this information but you may wish to correct the figure quoted which is grossly in error.

The Associated Octel Co. Ltd is the sole manufacturer and supplier of lead anti-knock compounds in the United Kingdom. The total production of lead alkyl compounds in 1972 was 101,000 tonnes, 27,600 tonnes of which were supplied to refiners in the United Kingdom and Eire, the remainder being exported worldwide. Since the lead metal content of anti-knock compound is approximately 40% w/w, the total weight of lead supplied to refiners in the United Kingdom and Eire was therefore around 11,000 tonnes in 1972.

Yours faithfully,

R. G. AICKIN

*The Associated Octel Co. Ltd,
London W1X 6DT*

Our figure for United Kingdom lead additives in petrol should have been 9,000 tonnes. Its appearance as 90,000 tonnes was a printing error—Editor.

news and views

Selectivity of nature

THE rate of evolution, and the varieties of adaptation open to a population depend on the degree of genetic variation which exists within it. The problem of measuring this crucial parameter seemed to be resolved with the use of the electrophoretic mobility of enzymes as markers for different alleles. This technique was first used to assess allele frequencies and heterozygosity in *Drosophila pseudoobscura* (Hubby and Lewontin, *Genetics*, **54**, 577; 1966) and has since been applied to numerous other species from armadillos to thrips. The results obtained from these vastly different species have been quite consistent. Between 10% and 30% of the gene loci sampled in a species are polymorphic, and 5%–15% of those in an individual are heterozygous. This seemed to provide abundant genetic variation on which selective pressure could act, until Kimura (*Nature*, **317**, 624; 1968) proposed that the different alleles at a given locus produce the same phenotype, so that most of the genetic variation revealed by enzyme electrophoresis is probably selectively neutral.

In that case, there would be comparatively little genetic variation which could be affected by evolutionary processes other than genetic drift. Kimura's argument for neutral mutation is based on the substitution rates of alleles producing macromolecules as the α and β chains of haemoglobin and cytochrome *c*, which have been roughly constant through evolutionary time. That would be the result if genetic drift were acting on neutral alleles, but not if there were alleles at a selective advantage or disadvantage. Kimura has further argued that the variation in fitness necessary to maintain so much genetic variability by selection would represent a "genetic load" so great as to result in the extinction of the population.

But heritable variability in virtually all characters has been the foundation of Darwinian evolution, and finding it difficult to believe that much of the genetic variability does not manifest itself in phenotypic variation, population geneticists have challenged Kimura's hypothesis on a number of grounds. Most of the parameters (effective population size, selection coefficients, mutation rates, and so on) necessary for population genetics theory are, however, unknown. This has meant working from 'reasonable' values to explore the consequences of the theory and see if plausible conclusions are reached; and what seems reasonable to one worker may not seem so to another.

Some more recent challenges have adopted a new approach, questioning Kimura's theory on its internal consistency in explaining certain kinds of data. There are several levels of attack, all based on the idea that even though relevant parameters such as population size are unknown, they have the same values for different loci of a species or for different alleles at a locus. Although, therefore, it is impossible to determine the selection acting on a specific locus or allele, it is possible, in some cases, to determine that there is selection on some of a group of loci or on some alleles at a locus. These consistency arguments, when properly applied, have been able to show that selection is acting on at least some of the loci which can be studied with electrophoretic techniques.

For example, if genetic drift is the only factor affecting gene frequency, each locus should be affected in the same way, regardless of the breeding structure—that is the population size, migration and inbreeding patterns—which are always unknown for natural populations. By sampling from several populations, it is possible to compute for each locus an effective inbreeding coefficient, F , which is nearly 1 when genetic drift is weak and nearly 0 when genetic drift is very strong. The observed F values for different loci can then be compared to find whether they are too heterogeneous to be consistent with neutral mutation theory. This procedure was first suggested by Cavalli-Sforza (*Proc. R. Soc.*, **B164**, 362; 1966) with data from human blood groups and later used by Lewontin and Krakauer (*Genetics*, **47**, 175; 1973) on the fly *Dacus*. The most complete study of this type is that of Nevo (*Nature*, **244**, 575; 1973), who sampled thirty-one loci in eleven populations of *Theomomys talpoides* (a pocket gopher). He argues that his discovery of F values ranging between 0 and 0.8 reveals too much heterogeneity to be consistent with neutral mutation theory.

Another approach, introduced by Johnson (*Nature*, **237**, 170; 1972) and analysed in detail by Johnson and Feldman (*Theor. pop. Biol.*, **4**, 209; 1973) is based on the evenness of the distribution of allele frequencies at different loci. If all of a given number of alleles at one locus are assumed to be neutral, then their frequencies are not expected to be either too similar or dissimilar. Each locus with a given number of alleles is an independent trial. Johnson and Feldman analyse previously published data on several *Drosophila* species and show that the frequencies are too even to be consistent with the neutral mutation theory. As with the distributions of inbreeding coefficients, one cannot determine which of the alleles are selected but only that there must be some factors, other than mutation and genetic drift which are affecting the gene frequencies.

Measures of the similarity of allele frequencies in different geographic regions forced Bulmer to the same conclusion (*Nature*, **241**, 199; 1973). Bulmer claimed that geographically separated natural populations of *Drosophila* are too similar in genetic composition for genetic drift, alone, to be acting. Maruyama and Kimura (*Nature*, **249**, 30; 1974) point out that Bulmer's argument depends on a theory which assumes implicitly that there are an infinite number of subpopulations and hence an infinite number of individuals in the species. Although mathematically correct, Bulmer's application was inappropriate. Maruyama and Kimura apply the theory of genetic drift in a finite number of subpopulations, first worked out by Maruyama (*Genetics*, **69**, 377; 1972), and conclude that the data are, in fact, consistent with the neutral theory. Although Maruyama and Kimura are forced to assume 'reasonable' values for several unknown parameters, they do show that, contrary to Bulmer's conclusion, the existing data on geographic variation are not inconsistent with the neutral theory. They also show, surprisingly, that even a very small amount of migration between subpopulations can maintain genetic uniformity in unselected loci over very large distances, on the same scale as the total range of the species. The next logical step will be to examine geographic variation in a single species in the light of Maruyama's theory in order to distinguish the selected from the neutral loci.

The recent challenges to the neutral mutation theory indicate a maturation of population genetics theory. Much of

the previous work in the subject has been directed to what might happen in natural populations in different sets of hypothetical circumstances. The apparent goal was to show that Darwinian evolution could be accounted for by changes in gene frequencies. Much of the recent theory takes that for granted and is motivated by and directed to observations of gene frequencies in natural populations. The neutral mutation hypothesis of Kimura has been one focus of such efforts. Although the studies of Nevo and Johnson show that some selection must be acting, their tone indicates the extent to which the neutral theory has been accepted. The question is no longer whether all of the genetic variation is selected or not but what fraction is selected. The recent work has shown that a significant fraction is selected, but it would be difficult to argue that the fraction approaches one.

From a Correspondent

How special is the Universe?

AN examination of the delicacy of advanced life forms and the requirement of highly special conditions for their existence would seem to support the long-standing tradition that the Universe has been created in a singularly convenient form for the presence of intelligent life.

It is truly remarkable that modern theoretical physics can make a contribution to this philosophical question. For example, it is sometimes pointed out how various naturally occurring numbers must be restricted in their values in order to be consistent with the existence of living matter. Dyson has remarked (*Scient. Am.*, **225**, 51-59; 1971) that an increase in the coupling constant of the strong interaction by only a few percent would have resulted in a catastrophic synthesis of most of the hydrogen in the Universe during the early stages of the big-bang, thereby depriving stable stars such as the Sun of their energy source, making life seemingly impossible. Another important naturally occurring number is the ratio between the electrical and gravitational forces between an electron and a proton (a staggering 10^{39}). Strangely, this number coincides with the age of the Universe measured in atomic units of time. Carter has argued (unpublished work) that this coincidence is necessary for the existence of intelligent life, on the basis that the lifetime of stable stars depends on the first number, and that this must be somewhere near the time required for intelligent life to evolve, which is of course just equal to the present age of the Universe. Thus the approximate equality of the two numbers is not a coincidence, but a direct result of our own existence.

The application of this philosophy to the initial state of the Universe rather than to the values of the constants of nature has been made by Collins and Hawking (*Astrophys. J.*, **180**, 317-334; 1973) who addressed themselves to one of the outstanding mysteries of modern cosmology. Why, out of all the possible distributions of matter, are we living in a Universe in which this distribution is so accurately isotropic? For it is a remarkable fact that whichever direction we look, the 3 K background radiation has the same intensity to one part in 1,000. This fact is all the more puzzling when it is realised that such widely separated regions of the Universe cannot have even been casually connected when this radiation was emitted, owing to the existence of a so-called particle horizon across which no information can pass. Collins and Hawking have pointed out, however, that although the Universe appears isotropic now, it need not always have been so. Restricting their discussion to homogeneous cosmological models, they show that there is a class of cosmologies which become isotropic as they expand from the initial big-bang. The most relevant discovery of their work is that the set of initial states of the Universe for this

to happen is of measure zero in the set of all possible initial conditions. In layman's language, there can be no deviation at all from these special initial states if the Universe is to become isotropic. Moreover, it is just this same class of cosmological models which permits the growth of galaxies and stars by ordinary gravitational condensation, and hence permits the existence of life. So Collins and Hawking conjecture that man's own existence is a consequence of the isotropy of the Universe (see *Nature*, **242**, 304; 1973).

It must not be supposed that the adoption of the philosophy that the existence of life imposes severe constraints on the structure of the Universe implies a 'purpose-built' world with man as the end product. Most subscribers to the former viewpoint prefer to conjecture that there is not just one single Universe, but a whole ensemble, all with widely varying properties. In most of these universes life would be impossible because the very restrictive conditions would not be met. But, in some like our own, intelligent life is able to evolve and become conscious of its surroundings—in a nutshell, the world we live in is the world we live in. In answer to the question—where are these other universes?—we have two choices. Wheeler has suggested that our own Universe may not continue to expand forever, but may be returned eventually to a very dense condition where it will be 'reprocessed' and emerge into another cycle of expansion and recontraction. These cycles are supposed to continue indefinitely, passing through an infinite variety of structure and conditions with the 'constants' of nature, and perhaps even the laws of physics changing from one cycle to the next. Only very rarely does a cycle permit conscious organisms to evolve. Another possibility has been suggested by Everett in his interpretation of the laws of quantum mechanics. It is well known that quantum theory cannot predict precisely the outcome of any realistic experiment, but at best can only give the relative probabilities of the various possible outcomes. Conventionally, it is supposed that the real world selects from all the choices one particular result at random, but Everett has developed a theory in which all possible quantum alternatives presented are in fact realised, by the entire world splitting continuously into a stupendous number of 'parallel worlds'. Once again, only a small subset of such worlds would permit the existence of life.

Curiously enough, this 'bioreselection' philosophy is usually accredited to modern cosmologists such as Dicke, Carter and Wheeler. In fact it was also used as long ago as the last century by Boltzmann in an attempt to explain why the Universe was in such a very remarkable state, rather than the much more probable condition of thermodynamic equilibrium. Boltzmann proposed that the Universe indeed spent nearly all its time in equilibrium, but on exceedingly rare occasions produced a vast fluctuation from equilibrium, thereby explaining its present (unstable) condition. The reason why we happen to be here to observe this extraordinarily unlikely occurrence because it is itself a necessary precondition for life, which requires continual thermodynamic disequilibrium for its existence. Boltzmann's fluctuations, like Wheeler's cycles, were supposed to occur repeatedly, though only after enormous durations.

Popular though this bizarre philosophy may be, there exists a counter philosophy the proponents of which are no less distinguished. According to this latter point of view, the Universe is not at all a remarkable place, but the almost inevitable outcome of a very wide range of initial conditions. Thus, for example, it has been claimed that there are various dissipative effects which would be present in the early Universe, and have the effect of damping out any anisotropies which may have been present initially, thereby leading to the high degree of isotropy now observed. In particular, Misner has examined the effects of neutrino viscosity, and recent exciting work by Zel'dovich and Starobinskii in the Soviet Union has shown that the tidal

gravitational effects present in an initially anisotropic Universe would give rise to creation of particle pairs out of the vacuum. The back reaction of this particle production on the evolving Universe is such as to remove some of the isotropy. Whether or not processes like these can really explain the isotropy of the Universe is not yet known, and it is in any case hard to see how sufficient damping could work for any initial conditions.

The most recent contribution to this fascinating debate is published by Bonnor in the latest *Monthly Notices of the Royal Astronomical Society* (167, 55–61; 1974). One of the restrictions of the Collins–Hawking result was that they treated only homogeneous cosmological models, on the supposition that any inhomogeneity would be expected to impede rather than assist the growth of isotropy. Bonnor claims, however, that this supposition may be unfounded. By removing the assumption of homogeneity and investigating the evolution of cosmological models which are initially inhomogeneous, he finds that under a wide range of initial conditions these models eventually approach both homogeneity and isotropy, that is, the Robertson-Walker type universes. Unfortunately, it is not clear from Bonnor's work just how general this result is, for his model is also restricted by being spherically symmetric about a privileged point in space, and therefore does not obey the usual cosmological principle.

The significance of Bonnor's result for the philosophical debate is that our present isotropic Universe may well have evolved from one of a wide variety of initial states of varying inhomogeneity, rather than from the exactly determined class of Collins–Hawking models. Whatever the outcome of this continuing debate, it is bound to have an influence on the way in which man relates himself to the Universe.

P. C. W. DAVIES

Changing views of the Moon's magnetism

BEFORE 1959 there was no direct way of telling whether the Moon possesses a magnetic field or not. But in that year prospects for the experimental determination of any possible lunar field improved markedly when Luna 2 took a magnetometer down to within 55 km of the Moon's surface. No field was actually observed, which, taking into account the sensitivity of the magnetometer, put an upper limit of 6×10^{21} gauss cm³ on the Moon's global magnetic moment—a maximum value some four orders of magnitude lower than the present dipole moment of the Earth. This apparent lack of any significant global magnetic field on the Moon was confirmed a few years later by Luna 10, and again in 1967 by Explorer 35 which enabled the maximum moment for a dipole aligned along the lunar axis to be reduced to 6×10^{20} gauss cm³ (Ness *et al.*, *J. geophys. Res.*, **72**, 5769; 1967 and **73**, 3421; 1968). A more detailed analysis of the Explorer 35 data, carried out a little later by Behannon (*J. geophys. Res.*, **73**, 7257; 1968), yielded a still lower value of 1×10^{20} gauss cm³, and the most recent estimate of the maximum lunar dipole moment (based on Apollo subsatellite magnetometer results) is 6×10^{19} gauss cm³.

Thus for more than a decade from the first attempt to determine the lunar magnetic field directly, most (but not quite all) indications were that the Moon is magnetically inert—a result entirely in accord with preconceived ideas about the nature of magnetism in planetary bodies in general and about the nature of the Moon in particular. Because the only viable theory for the origin of the Earth's highly variable magnetic field involves dynamo action in the molten core, it seemed natural to consider extraterrestrial bodies in similar terms. In other words, the absence of a global field on the Moon was seen as the result of the lack

of any dynamo process in the lunar interior; and the absence of a lunar dynamo was to be expected, partly because the existence of molten metallic core of appreciable size seems to be precluded and partly because the Moon's rate of rotation is low. So few people were surprised at the inability to detect a lunar global field. It is true that before the end of the 1960s some of the Explorer 35 data had also been interpreted as indicating the existence of small scale (10–100 km) magnetic anomalies on the Moon, mainly in the highlands. But although the presence of anomalies has since been confirmed, the original interpretation was indirect and attracted comparatively little attention. It is thus historically quite correct to suggest, as Fuller (*Rev. Geophys. Space Phys.*, **12**, 23; 1974) does in his excellent review of the subject, that before the Apollo programme the Moon was generally regarded as 'magnetically uninteresting'.

Against this background of assumption and self-consistency, it apparently came as quite a shock to many geophysicists to discover that some of the returned Apollo 11 samples contained stable natural remanent magnetisations (NRMs) acquired before leaving the Moon—a discovery repeated with samples from subsequent Apollo missions. Moreover, magnetometers landed on the Moon by Apollo 12 onwards recorded surface magnetic fields in magnitude from about a gamma to hundreds of gammas; and there can be no doubt that these fields arise from the magnetisations of rocks in the lunar surface. Understandably enough, there was again a tendency to see these new and unexpected results in terms of processes comparable to those occurring on Earth. Thus for one thing it seemed reasonable to suppose that the NRMs in at least the lunar igneous rocks were thermoremanent magnetisations (TRMs) acquired as the rocks cooled through their Curie points. Since the time span by the magnetic lunar rocks represents at least several hundred million years, the implication then is that the magnetising field must also have lasted that long; and the field source most compatible with a long-term existence is, of course, the dynamo. In other words, although the Moon now has a dipole moment, if any, of less than about 10^{20} gauss cm³ and apparently does not support a dynamo, for an extended period over 3,000 million years ago it must have acted as a dynamo giving rise to a dipole moment much greater than 10^{20} gauss cm³.

Clearly this chain of argument contains its own inbuilt assumptions, the most obvious of which is that during its early history the Moon must have possessed a molten core with properties compatible with dynamo action. Presumably the lunar interior then cooled or the Moon's rate of rotation decreased, or both, so that at some time during the past 3,000 million years or so the dynamo ceased. All this is quite possible, although as Strangway and Sharpe point out on page 227 of this issue of *Nature*, as a theory it is rapidly going out of fashion in favour of one involving a Moon which accreted cold and subsequently heated up. This rules out an early lunar dynamo, but as Runcorn and Urey (*Science*, **180**, 636; 1973) noted last year, opens up the possibility, long rejected in the case of the Earth, of a magnetising field derived from permanent magnetism. In other words, although rocks such as the mare basalts would still have acquired their magnetisations thermally, they would have done so not in a field of dynamo origin but in the field from a 'permanently' magnetic lunar interior. As this permanent magnetism evidently does not exist now, it was presumably destroyed thermally as the Moon gradually heated.

By what process and in what type of field the permanent magnetism itself formed are not known. There is no shortage of fields suitable in principle (a much stronger solar field, a much stronger solar wind field, or the terrestrial field when the Moon was much closer to the Earth, for example), although it is difficult to see how the one which actually

magnetised the Moon can ever be identified. As far as the lunar magnetisation process is concerned, Run-corn and Urey rejected TRM because the required sequence of temperature changes is difficult to envisage and rejected viscous magnetisation because of its fundamental instability. Instead, they favoured depositional magnetisation involving magnetised particles settling nonturbulently through the accreting gas sphere in the external magnetic field, a process consistent with the existence of low temperatures. In this issue of *Nature*, Strangway and Sharpe now investigate a fourth possibility—iso-thermal magnetism acquired in a steady external field—in the light of the Moon's thermal evolution.

PETER J. SMITH

Dimensions of the possible

David Davies

THE "Recognition of Alien Life", a symposium organised by N. W. Pirie (Rothamsted Experimental Station) at the Royal Society on May 2, could as well have been called the Carl Sagan Symposium. Sagan—from Cornell University—jetted in to London, talked to the press, gave the first lecture, was never far from other speakers' minds for the rest of the day, interjected wittily, moved on to lecture elsewhere and jetted out. And with good cause was he at the centre, for nobody can have thought more than he about life elsewhere.

Alien life may be intelligent or not; Sagan considered the intelligent sort. At present, he said, nothing is known about extraterrestrial intelligence, but the technology is now available for the analysis of signals from stars and the scanning of planetary surfaces. Critical experiments, in his view, are coming up soon. The best data with which to start are observations of the Earth such as, say, Martians could make (not that Sagan has much time for Martian intelligence). All depends on resolution, as a collection of satellite photographs show. At 1 km resolution he could find no sign of life on Earth—except at Cochran, Ontario! True, cities appear as dirty smudges, but they show no sign of reworking. A useful guide is 'departure from thermodynamic equilibrium' such as is shown by geometrisation of Earth features. At 100 m resolution, this begins to show up in, for instance, large field patterns. The nocturnal light of cities is obvious, but an observer would have doubts, as many large cities are on waterfronts; maybe some chemical process could generate the light.

Eavesdroppers at stellar distances on radio emissions from Earth would be

struck by the enormous energy density around metre wavelengths from radio, radar and television. These signals would be detectable at tens of light years and intelligent terrestrial life would rapidly be inferred from their highly ordered nature. With radio telescopes the range could be a thousand times greater, though given limited facilities, it is better, Sagan believes, to receive than transmit as man is likely to be the backward partner. The art of anticryptography—devising self evident messages for other intelligent life—is growing.

C. H. Waddington (University of Edinburgh) also considered intelligent alien life in trying to pin down what tells human beings that an artefact is just that. Unless one stumbles across a mechanical device, it is probable that repetition of shapes is as good a guide as any to intelligence. P. H. Gregory (Rothamsted) stressed that in talking about the recognition of life at the microscopic level the decision is largely an aesthetic one; on Earth man is still inadequately aware of the realms of possibility. He proposed an atlas of terrestrial microscopic objects as a valuable step towards improving methodology.

At this stage the meeting became philosophical as the discussion circled inconclusively round the question from the floor. "What is life?", reminding me of Gluck, my neighbour of Shelley and Sir Peter Medawar of flogging a dead horse.

The next contribution, by J. E. Lovelock (University of Reading), was in many ways the most fascinating. What could astronomical observations reveal about the probable existence of a biosphere? Thermodynamic disequilibrium in the atmosphere could be a good guide to the existence of life, and on Earth gross departures from equilibrium in nitrogen, hydrogen and methane point to life being present, a conclusion which multiplex spectrometry does not endorse for other planets.

The remainder of the meeting was devoted to questions of life on Earth, its past record, its energy relationships and its evolution. I. R. Kaplan (University of California, Los Angeles) reviewed stable isotope analysis, particularly of hydrogen, carbon, oxygen, nitrogen and sulphur. Variations in concentration ratios by biological enrichment are helpful in understanding past terrestrial environments. This scheme was taken further by P. C. Sylvester-Bradley (University of Leicester) for the Precambrian geological record. The origin of life is placed at about 4,000 million years ago (a review of this field appeared in *Nature*, 248, 730; 1974). J. De Ley (State University, Gent) discussed

energy sources and storage and remarked on the adenine-based nature of life on Earth through the dominance of ATP as an energy carrier. Finally, A. G. Cairns-Smith (University of Glasgow) speculated on the role of clays as possible catalysts or frameworks for the evolution of biochemicals from inorganic origins.

Medawar summed up characteristically: "The heterogeneity of life and the varieties of metabolism and modes of behaviour are so enormous that nothing should surprise us about alien life. All we can do is attempt to sketch out the dimensions of the possible".

Alloy glasses and beyond

from a Correspondent

At the eighth International Thin Films Conference held at the University of Sussex from April 8 to 10 under the sponsorship of the Thin Films and Surfaces group of the Institute of Physics, the most noticeable feature was the diversity of topics discussed: many of the phenomena of solid state physics have now been reproduced in thin film form, and for good measure some novel phenomena have been discovered.

The growth in the technology of electron-optical instruments continues to provide new and valuable aids to the structural investigation of thin films. According to M. Prutton (University of York), it seems that the scanning Auger spectroscope may soon become a viable research tool, and already it can be used to analyse the composition of a 10 Å-thick surface layer. In the scanning mode of operation, simultaneous composition analyses can be obtained from a large number of positions on the surface of a given specimen.

J. G. Wright and P. K. Leung (University of East Anglia) used *in situ* electron diffraction methods to observe thin films of amorphous Co, Fe, Mn and Cr prepared by evaporation under ultra-high vacuum onto a substrate at 4 K. This work should provide final confirmation that amorphous metallic films are not caused simply by impurities in vacuum systems. In a similar vein, B. Cantor and R. W. Cahn (University of Sussex) described the structures of highly extended solid solutions in binary metal alloy films.

A large section of the conference was devoted to various electrical and magnetic properties of thin films. C. H. L. Goodman (Standard Telephone Laboratories, Harlow) pointed out that previous claims that chalcogenide and metal oxide glasses represent the biggest breakthrough since the transistor

are exaggerated, and he was not very confident of their potential usefulness except in relatively limited areas such as addressed display systems. In a stimulating paper, N. Boonthanom and M. White (Brighton Polytechnic) described the co-evaporation of metal/polymer mixtures to form a structure of conducting metal islands in an insulating polymer matrix. In these films the resistivity changes by seven orders of magnitude over a temperature range of only 200 K.

The final session was concerned with the problems of fabricating magnetic bubble materials. It is a sign of the rate of progress in thin film physics that in such a recent development, the fundamental physics is already well established and the current problems are technological in nature.

Nutrient supply and ecosystem growth

from Peter D. Moore,
Plant Ecology Correspondent

THE term 'oceanicity' traditionally has conveyed to ecologists the impression of a climate with a low amplitude fluctuation in seasonal temperature and a high annual precipitation. There is now evidence, however, that other aspects of maritime environments—for example, nutrient cycling—may be of equal or greater ecological importance.

Nutrient elements in terrestrial ecosystems are cycled constantly between non-living and living components. There are gains and losses: gaseous elements are gained and lost through atmospheric movements; non-gaseous elements enter terrestrial ecosystems largely by means of the weathering of rock particles in the soil or as solutions or particulate fragments from the atmosphere, arriving as aerosol or dust. In some rather exceptional types of ecosystem other transport mechanisms may be important, such as inwash of detritus by rivers or sea, or the immigration of animals to roost, nest or defaecate. Non-gaseous nutrient elements are lost from ecosystems largely as a result of soil leaching (see Bormann and Likens, *Science*, **155**, 424; 1967).

In a stable, climax ecosystem nutrient gains are balanced by nutrient losses, whereas in seral ecosystems, still developing, gains exceed losses. The excess of gain over loss is normally associated with an increase in biomass. The final constraint which limits ecosystem development, however, is normally a climatic one rather than nutrient availability, although in some climax ecosystems (for example, ombrotrophic mires) nutrients may be limiting (Firbas, *Veröff. geobot. Inst. Zurich*, **25**, 177; 1952). It is possible, within the

More muonless neutrino events

from a Correspondent

LAST year the users of the European bubble chamber Gargamelle, at CERN Geneva, reported that they had observed neutrino interactions without outgoing muon tracks (*Phys. Lett.*, **46B**, 138; 1973; see also *Nature* **245**, 119; 1973). The simplest explanation for these events is that the weak interaction has a 'neutral current' component. If the effect is genuine then the implications are profound. The existence of a neutral weak current makes it possible to construct a rigorous theory combining both the weak and the electromagnetic interactions. Such theories have been proposed by Weinberg (*Phys. Rev.*, **D5**, 1412; 1972), and others.

When the Gargamelle result was announced there were unofficial reports that an American group had seen similar events in an experiment at the National Accelerator Laboratory (NAL) near Chicago. Now, after almost a year of careful checking, these data have been published (Benvenuti *et al.*, *Phys. Rev. Lett.*, **32**, 800; 1974) by a combined team from Harvard, University of Pennsylvania, Wisconsin and NAL. The groups used scintillation-counter and spark-chamber techniques to detect the events, to measure the energy of the reaction

products and to identify muon tracks. Their neutrinos were produced from primary proton beams of 300 and 400 GeV, much higher than the 30 GeV energy of the CERN proton beam.

The team saw 76 events which must have been caused by neutrino interactions, but did not have a muon track: 93 neutrino events were seen with an identification muon. A number of different techniques have been used to calculate how many muon events had been wrongly identified, in particular, how many muons missed the muon detector. All versions of this calculation fail to account for the whole of the observed signal of muonless events. About 36 genuine muonless events remain, giving a corrected ratio of muonless events to muon events of 0.23 ± 0.09 .

Since this ratio is almost the same as that seen by Gargamelle, even though the detection technique is totally different and the beam energies are ten times larger, there can now be no doubt that muonless neutrino events do occur. Whether they are caused by a neutral weak current is not yet completely certain, but other explanations are becoming increasingly implausible.

general climatic restrictions, that availability of nutrients in an ecosystem could influence the rate at which succession takes place and equilibrium is attained.

In the light of this knowledge of nutrient cycling in ecosystems, it is rather surprising that nobody has yet attempted to measure the rates of successions in maritime regions where aerial nutrient input can be expected to be high and to compare these with areas of similar edaphic and climatic qualities far removed from the sea. Art, Bormann, Voigt and Woodwell (*Science*, **184**, 60; 1974) have now produced data from a maritime climax forest ecosystem which makes such a comparison even more imperative.

Fire Island, the research site, is a barrier island off Long Island, New York. It is fringed by sand dunes and salt marshes and contains a forest ecosystem in the lee of mature dunes. Art and his colleagues have attempted to determine the source of incoming nutrients to this forest and, by a process of elimination, they have come to the conclusion that aerosols and airborne particles constitute most of the nutrient input. They consider weathering an unimportant source of nutrients because

the substrate is 98% quartz sand and the remainder (magnetite, garnet and tourmaline) is also unlikely to be a major source of ions. Animal inputs, mainly seabird guano, are only locally important and are of no significance within the study area. The soaking of seawater into the ecosystem from below does not occur because of the peculiar hydrology of the island; a high precipitation: evaporation ratio leads to a downward movement of freshwater to a depth of about 40 m below sea level in the centre of the island, thus excluding salt water. There remains the income from airborne sources.

Art *et al.* divide the airborne material into three types—a rain component (collected in rain gauges open only during rain), a dry fallout component (from gauges open all the time, less the rain component) and an impaction component (by measuring the difference between above canopy and below canopy composition of rainfall and determining the excess beneath canopy). Total annual figures of meteorological input obtained were (in g m^{-2}): 0.73 K; 14.15 Na; 0.98 Ca; and 1.91 Mg. These figures compare very closely with those obtained in other oceanic situations; for example, the Shetland Isles (see

Gore, *J. Ecol.*, **56**, 483; 1968) 0.55 K; 13.3 Na; 0.67 Ca; and 1.92 Mg and on the Lizard Peninsula in Cornwall (Malloch, *J. Ecol.*, **60**, 103; 1972) 3.8 K; 19.8 Na; 0.9 Ca; and 2.0 Mg.

The annual uptake of nutrients by the vegetation of the Fire Island forest was determined by measuring the nutrient content of the net primary production in midsummer. The loss of nutrients by litterfall and canopy leaching was found to balance this uptake for most elements, and it would thus seem that the forest is in a climax, equilibrium state. The limitations upon growth in this ecosystem seem to be quite complex. Increased nutrient input would, theoretically, result from a larger tree canopy (that is, greater above-ground-biomass) because this would enhance the impaction component, but this is made impossible by the destruction of emergent shoots by high intensity, salt-laden winds. Vegetative proliferation is possible only by low-level lateral branches in the canopy and this seems now to have reached maximum development; as a result a climax has been attained.

In terms of biomass ($17,000 \text{ g m}^{-2}$) and net primary productivity ($1,100 \text{ g m}^{-2}$) the Fire Island forest compares well with the figures for temperate forest collected by Rodin and Basilevich (in *Functioning of Terrestrial Ecosystems at the Primary Production Level* (edit. by Eckardt), Unesco, Paris, 1968). This is despite the extreme nutrient poverty of the sandy substrate. A comparison of nutrient input figures from the Fire Island forest with those from other forest ecosystems shows that the meteorological input alone of several important elements (for example, K and Ca) equals the total (meteorological plus weathering) input of these elements in forests further from the sea. Thus, if nutrient availability is arresting the succession, it is not surprising that the biomass of this 'subclimax' falls little short of that found in climatically limited forests.

Finally, to return to the relation between nutrient availability and the rate of successions, it is interesting to note that the Fire Island forest is considered, on the basis of radiocarbon dating and geological evidence (Sirkin, *Bull. Torrey bot. Club*, **94**, 131; 1972), to be only 200–300 years old. The dune systems around the inland lake Michigan are considered to have taken thousands of years to attain a forested state, but there the low nutrient availability from weathering is accompanied, one presumes, by a low aerial nutrient input. Much more data are needed from such inland dune systems in terms of their nutrient balance, but it seems that there is a strong case for supposing that overall nutrient availability is the factor controlling the rate of succession

on those sand dunes. Such a knowledge will undoubtedly provide ammunition for those ecologists who consider that the limiting effects of nutrient supply upon the developmental rate of ecosystems is a general feature of successions (see *Nature*, **240**, 443; 1972).

Repressors at the molecular level

from Stephen Neidle

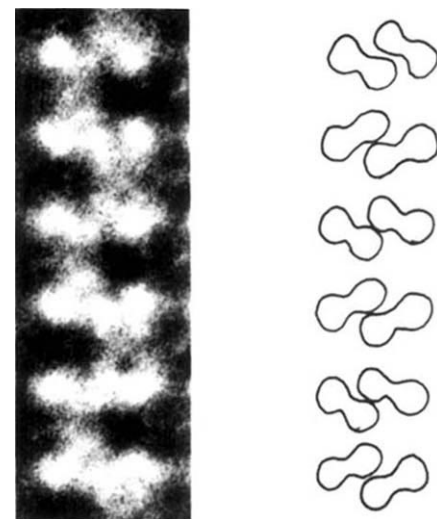
THE cluster of three genes responsible for lactose metabolism in *Escherichia coli* have been perhaps the most intensively studied of all bacterial chromosome systems. Indeed advances in knowledge of this cluster, known as the *lac* operon, have often tended to be hailed as landmarks in molecular genetics generally. It is now some 13 years since Jacob and Monod first proposed that the control of protein synthesis in bacteria involves certain agents that can repress the relevant genes, and hence inhibit specific messenger RNA synthesis. The first of these substances to be isolated and characterised was the lactose (*lac*) repressor, which is a tetrameric protein of molecular weight 150,000 (Gilbert and Müller-Hill, *Proc. natn. Acad. Sci. U.S.A.*, **56**, 1891; 1966). These workers followed this considerable technical achievement by demonstrating convincingly that the repressor does indeed act on the *lac* operator locus of a DNA molecule (*ibid.*, **58**, 2415; 1967).

Detailed knowledge of how repressor proteins turn off DNA at the molecular level, however, is still almost entirely lacking, although some interaction and control models have been proposed, notably by Sobell, Müller-Hill and Gierer. The amino-acid sequence of the *E. coli lac* repressor, as well as the nucleotide sequence of its target on the chromosome, the *lac* operator, have both been recently determined (*ibid.*, **70**, 3576; 1973). Thus, it is not altogether unexpected that protein crystallographers have begun to take an interest in repressor proteins, especially since modern techniques can now provide structural information about such large molecules. Steitz and his colleagues at Yale (*ibid.*, **71**, 593; 1974) have succeeded in crystallising the *lac* repressor, but have obtained disappointingly small microcrystals. Although these are not large enough for even preliminary single-crystal studies, they have revealed a measure of useful information. A combination of powder X-ray diffraction data and electron micrographs has given a good indication of the size and possible symmetry of the unit cell of the protein, as well as that of the tetrameric unit itself. There are four of these tetramers in

each orthorhombic repeat, and they appear to be asymmetric and dumbbell in shape, with a length of about 135 Å. It seems most likely that the subunits of the repressor molecule are related by D_2 point-group symmetry, rather than the four-fold symmetry proposed by Sobell (*ibid.*, **69**, 2483; 1972).

This necessarily meagre amount of data has nonetheless enabled Steitz *et al.* to both reject several of the proposed repressor–DNA operator interaction models, and to suggest one of their own. Any such model has to take into account the two-fold symmetry regions found in the operator nucleotide sequence by Gilbert and Maxam. Rather than binding to either single stranded DNA or four-leaved cloverleaf DNA (both suggested arrangements for the operator locus), the new suggestion is that the dumbbell repressor binds in a manner such that its long axis and the double-helical polynucleotide axis are parallel. The arrangement is described as being similar to a "hotdog in a hotdog bun", which analogy is perhaps clearest to those most familiar with the finer points of the American cuisine. The model has, however, several attractive features, notably that it does not contravene accepted dogma regarding protein–substrate and protein–protein interaction. Also, it has two-fold symmetrical binding grooves, which comply rather well with the operator sequence data.

Until there are more detailed structural studies of both the repressor protein itself, and maybe even complexed with the *lac* operator fragment of the genome, there will doubtless be numerous more suggested models.



Steitz *et al.*'s enlargement of part of the translationally averaged electron micrograph with, on the right, their interpretation of the molecular outline. Each dumbbell is one repressor tetramer and there are four tetramers per unit cell.

Roundtable on neuroendocrinology

from Barend ter Haar

DURING the weekend April 6-7 recent neuroendocrinological findings of most of the leading laboratories in Europe were discussed informally at Magdalen College, Oxford in an effort to establish the most fruitful course of future research, and to sow the seeds for greater international collaboration. The sessions were divided under the general headings of hypothalamic releasing hormones, secretion of the hormones of the posterior pituitary, monoamine systems affecting endocrine function, rhythms involved in neural control of reproductive function and processes involved in sexual differentiation and puberty.

It had been hoped that with the elucidation of the amino acid sequence of luteinising hormone releasing hormone (LHRH) and its subsequent chemical synthesis, antibodies prepared against this material could be used to identify, by immunofluorimetric techniques, the neurosecretory cells for LHRH in the brain. Though J. Barry (University of Lille) has been able to demonstrate a distribution of such neurones throughout certain areas of the anterior and medial hypothalamus of the guinea pig, he has as yet been relatively unsuccessful in other species: there is still much progress to be made in this field. It was noted with interest that Barry has also found labelled axons extending from the hypothalamus to the limbic system: it is now becoming more obvious that classical concepts of unidirectional flow of neuronal information from the limbic to the anterior hypothalamus and then to the median eminence have to be altered.

To add to the complexity, M. C. Harris (University of Birmingham) and G. B. Makara (Institute for Experimental Medicine, Budapest) indicated that they had also found not only cells in the arcuate nucleus and median eminence with axonal terminations in the anterior hypothalamus, but also hypothalamic cells which appeared to project both forwards, to the paraventricular nucleus, and also caudally, to the median eminence. Of further interest concerning efferents from the hypothalamus to other brain areas, and in the context of recent observations of effects of thyrotropin releasing hormone (TRH) on certain psychiatric disorders, were the observations presented by R. Dyer (University of Bristol) that both TRH and LHRH can modify electrical activity in the cerebral cortex in a similar way to their observations in the hypothalamus.

It is clear that though radioimmuno-

assay techniques have greatly helped to advance neuroendocrine knowledge, great care is still needed in the interpretation of results, the principal difficulty being that immunoreactive sites on a molecule do not necessarily relate to biologically active sites. Furthermore, such assay techniques are of little use in the detection of brief hormonal discharges such as the 2-second discharge of oxytocin demonstrated by D. W. Lincoln (University of Bristol) by means of transient changes in mammary pressure. For the release of posterior pituitary hormones, N. A. Thorn (University of Copenhagen) suggested a similar involvement of calcium in hormone discharge as in the mechanisms of muscle contraction.

In discussing the effect of circadian rhythms on reproductive function, B. K. Follett (University College of North Wales, Bangor) described how in the sparrow photosensitivity seems to vary in a circadian fashion in that a limited period of sensitivity occurs about 12 h after dawn. He has found that if light is present at this time reproductive activity is promoted so that sexual activity becomes linked with maximum day length. P. C. B. MacKinnon (University of Oxford) described how the circadian rhythm of cerebral protein metabolism in the rat is sexually differentiated, and put forward the hypothesis that the 'female' rhythm is involved in the synchronisation of internal and external signals to bring about reproductive activity in the most suitable conditions.

In considering the processes involved in the onset of puberty, F. Döcke (Humboldt University, Berlin) indicated that the limbic system is involved in controlling sexual development, and H. Moll (Erasmus University, Rotterdam) suggested that the search for only one control process may be misguided, and that puberty may be brought about by the coordinated maturation of several processes.

Obviously much more was discussed than there is space here to relate. The organisational efforts of B. T. Donovan (Institute of Psychiatry, London), C. Kordon (INSERM, Paris), and G. P. van Rees (University of Leiden) in getting together so many authorities on various branches of neuroendocrinology were well rewarded by the informal exchange of ideas that was achieved.

Correction

In the News and Views article "Beyond systems ecology" (*Nature*, **248**, 637; 1974), the authorship of the two publications cited was misattributed. The article in *Proc. N.Y. Acad. Sci.* is by R. Levins, and the book *Stability and Complexity in Model Ecosystems* is by R. M. May.

Characterising the stringent system

from Benjamin Lewin
Molecular Genetics Correspondent

POSSIBLE relationships between protein synthesis and transcription have attracted attention for more than a decade, and in recent months this concentration of interest has been justified by results which may lead to the definition of the components of the stringent system of *Escherichia coli*. Genetic characterisation of this system has so far led to the isolation of two classes of mutant. As was first shown by Cashel and Gallant (*Nature*, **221**, 838; 1969) and Cashel (*J. biol. Chem.*, **244**, 3133; 1969) relaxed (*rel*⁻) mutants do not restrain ribosomal RNA synthesis during starvation for required amino acids because (unlike the wild type stringent cells) they cannot increase their levels of the 'magic spot' compounds ppGpp and pppGpp in response to the deprivation. Cells of the spotless (*spoT*⁻) mutant, isolated more recently by Laffler and Gallant (*Cell*, **1**, 27; 1974), cannot produce pppGpp during amino acid starvation, but overproduce ppGpp.

Although it is clear that accumulation of ppGpp and pppGpp is responsible for inhibiting RNA synthesis, the mechanism of formation and action of these two compounds has only more recently come under investigation. By following the abilities of *rel*⁺ and *rel*⁻ strains to form magic spots and respond to changes in growth conditions, Lazzarini, Cashel and Gallant (*J. biol. Chem.*, **246**, 4381; 1971) found that *rel*⁻ cells, although unable to respond to amino acid starvation, show the same response as *rel*⁺ cells to other changes, such as shifts in growth rate. The *rel* gene must therefore code for a protein implicated in forming ppGpp and pppGpp specifically in response to lack of amino acids, a reaction which has proved to be mediated by the consequent lack of aminoacyl-tRNA. By showing that the stability of ppGpp is much enhanced in *spoT*⁻ cells, Stammers and Lazzarini (*Cell*, **1**, 85; 1974) have come to the conclusion that the *spoT* gene product may be responsible for converting ppGpp to pppGpp. Clearly, many other genes may also be part of this system and the levels of the magic spots may be controlled at degradation as well as synthesis.

The concept originally suggested some time ago that an 'idling reaction' of protein synthesis may be responsible for formation of magic spots has recently been substantiated by the use of *in vitro* systems. Haseltine *et al.* (*Nature*, **238**, 381; 1972) found that ribosomes from stringent cells can synthesise ppGpp and pppGpp *in vitro*

from substrates of GTP and ATP; and ribosomes of relaxed cells are unable to perform this reaction, but can do so when extracts are supplemented with a 'stringent factor' extracted from wild type cells. Formation of the magic spot compounds takes place *in vitro* when the A site of the ribosome is occupied by an uncharged transfer RNA; this evidently acts as a signal to the ribosome that protein synthesis is limited and allows the cell to titrate its amount of uncharged tRNA.

The protein responsible for responding to the blocked A site has been studied by Ramagopal and Davis (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 820; 1974). Examining *rel⁺* ribosomes showed that the protein is easily extracted from them and so may be only loosely bound to the ribosome. Using an assay in which protein extracted from *rel⁺* ribosomes was measured for its ability to stimulate the reaction *in vitro* of unwashed *rel⁻* ribosomes (the lack of washing means that these ribosomes possess all other necessary components), Ramagopal and Davis found that 50S subunits, 70S ribosomes of polysomes, and 70S ribosomes which have runoff polysomes all possess *rel⁺* protein activity. The protein activity is not present on 30S subunits. These results suggest that the stringent factor is a ribosomal protein as such, rather than a factor which associates with the ribosome during only part of its protein synthetic cycle, and is a loosely bound component of the 50S subunit. Since it is present on both free and polysomal 70S ribosomes, these results cast no light on the conditions necessary for formation of magic spots. But they offer the prospect that further characterisation of the protein may allow both its stringent activity and also whatever other functions it may have to be defined.

Although both genetic analysis and biochemical characterisation of the stringent system suggest the outlines of its operation, many important questions remain to be answered. The first class concerns the formation of ppGpp and pppGpp. Clearly, they are formed on the ribosome in response to inhibition of protein synthesis by entry of uncharged tRNA to the A site. Although the exact mechanisms of this reaction remain to be elucidated, the *rel⁺* protein seems to mediate this response. One discrepancy which remains to be resolved is that ppGpp can be formed *in vitro* from GDP and ATP, whereas pppGpp can be synthesised from GTP and ATP; but *in vivo* the *spoT* gene product has been implicated in converting ppGpp to pppGpp. Another critical reaction, so far investigated scarcely at all, is how these components are formed in response to conditions other than amino acid starvation—

isolation of mutants in these processes must provide an essential first step in analysis.

The second set of problems, which at the present seems more difficult to approach, concerns the reactions by which ppGpp and pppGpp mediate the inhibition of rRNA synthesis. One particularly interesting problem is why there are two magic spot compounds and what difference there may be in their functions. But attempts to show *in vitro* that ppGpp influences rRNA synthesis at the level of RNA polymerase activity have not so far proved very satisfactory. Yet the nature of this general coordinating system may prove to throw an important new light on the control of transcription in bacteria.

Control of 3T3 cell growth

from Brigid Hogan

THE mouse 3T3 line—so-called because it was derived from embryo cells transferred every 3 days at an inoculum of 3×10^5 —since it was established in 1963 has been widely used in studies of the control of proliferation of mammalian cells in culture. As long as the strict transfer regime is maintained, the cells consistently show distinct growth properties. First, they are very sensitive to a reduction in serum concentration in the medium and divide about five times slower in 1% than in 10% serum. Second, even if the cells are regularly replenished with 10% serum medium, they only grow very slowly after reaching a monolayer density of about 5×10^4 cells per cm^2 . Finally, they will not grow in suspension in agar or methylcellulose. It is, however, well established that 3T3 cells transformed by the oncogenic viruses SV40 or polyoma exhibit very different properties; they divide rapidly in 1% serum, reach far higher densities and form colonies in agar.

During the past few years Pollack's group in Cold Spring Harbor has been investigating the complex interrelation between these three parameters and the way in which they are modified by tumour viruses, by selecting sublines of SV3T3 which have reverted back to more normal behaviour. The first lines were selected for a reduction in growth at high cell density by exposing dense cultures to 5-fluorodeoxyuridine for 2 days so that cells synthesising DNA were killed. Clones which survived this treatment had a much more 'normal' morphology and were therefore called "flat" revertants, and reached a saturation density of only about 8×10^4 per cm^2 , although the shutoff in division at this density is not as complete as in normal cells.

More recently similar 'density revertants' have been selected by killing growing cells with 5-bromodeoxyuridine or colchicine (Vogel *et al.*, *J. cell. Physiol.*, **82**, 181-188; 1974). When their other properties were tested, the density revertants fell into two classes; one which grew well in 1% serum and one which did not, but neither class would grow in agar. Using similar techniques Pollack's group have also selected 'serum revertants' which do not grow rapidly in low serum (Vogel and Pollack, *ibid.*, 189-198); these also show low saturation density but may or may not grow in suspension. Thus it has been possible to select variants of SV3T3 which have reverted in either of two, or in all three aspects of transformation. All classes of revertants have more chromosomes per cell than the parent, synthesise SV40 Ts antigen, and in some cases have been shown to contain intact virus genomes, so that their altered behaviour is likely to arise from a change in the expression of some cellular gene(s).

Pollack's group have not been slow to take advantage of the properties of their mutants to throw light on the role of cyclic AMP in the control of 3T3 cell proliferation (Oey *et al.*, *Proc. natn. Acad. Sci., U.S.A.*, **71**, 694-698; 1974). In agreement with many other groups they found that sparse cultures of 3T3 in 10% serum contain twice as much intracellular cyclic AMP as SV3T3s. In the same conditions most revertant lines returned to having the higher levels of cyclic AMP characteristic of 3T3s, but two retained the low concentration found in SV3T3s. These two are the only revertants able to form colonies in methylcellulose so that there may be a correlation between low amounts of cyclic AMP and ability to grow in suspension. Only those revertants which are unable to grow in low serum share the property with 3T3s of rapidly and extensively increasing their concentrations of cyclic AMP when the serum concentration is reduced to 1%, and density revertants which can grow in low serum maintain the lower concentrations of cyclic AMP found in SV3T3s.

Finally, Oey *et al.* allowed normal 3T3s and two serum revertants to attain their low saturation density in conditions in which the 10% serum medium was regularly replaced, even after cell growth had slowed down. In all three lines they failed to find an increase in cyclic AMP as the cells reached their saturation densities. Taken together with the other findings, this suggests that density restriction and serum restriction of growth are not identical phenomena, as some workers have postulated, but are mediated by two different, but probably inter-related, mechanisms.

Radioactivity of tobacco trichomes and insoluble cigarette smoke particles

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Airborne ^{210}Pb is concentrated on small Aitken particles which accumulate on tobacco trichomes. Tobacco curing and the combustion of trichomes in burning cigarettes produce insoluble particles of high ^{210}Pb radioactivity which are inhaled and deposited in the bronchi of smokers. The subsequent ingrowth of ^{210}Po results in high local α irradiation which may account for bronchial cancer among smokers.

EXPOSURE to α radiation from the short-lived radon daughters, ^{218}Po and ^{214}Po , is generally considered the cause of bronchial cancer in uranium miners¹. The presence of the α emitter ^{210}Po in the bronchi of smokers in excess of ^{210}Po levels of non-smokers^{2,3} thus warrants serious consideration as the possible agent of bronchial cancer among smokers. Both ^{210}Pb and ^{210}Po have been observed in tobacco, in cigarette smoke and in smokers' lungs²⁻⁷.

Radford and Hunt⁴ pointed out that volatile ^{210}Po compounds are inhaled by smokers and suggested that ^{210}Po was bound strongly to the surfaces of smoke particles, thereby entering the bronchi. But, as Holtzman⁸ and others pointed out, it is likely that the volatile compounds of ^{210}Po which are inhaled are widely dispersed and cleared rapidly from their deposition sites in the lung. Little *et al.*², however, found high local tissue concentrations of ^{210}Po corresponding to an estimated maximum dose of ~ 200 rem per 25 yr to the bronchial epithelium of lower-lobe bifurcations of cigarette smokers⁹. This can be explained by the accumulation of insoluble ^{210}Pb particles in the bronchial bifurcations. The ^{210}Pb activity of insoluble smoke particles is sufficiently high to give rise to α doses much higher than 200 rem per 25 yr in regions of high particle concentration.

^{210}Pb on tobacco trichomes

Reports on the origin and distribution of ^{210}Pb and ^{210}Po in tobacco plants are contradictory. Tso *et al.*⁹, conclude that the principal mechanism involves uptake into roots from soils and fertilisers, whereas Francis *et al.*¹⁰ suggest deposition in rainfall. The properties and distribution of trichomes (hairs) on tobacco leaf surfaces^{11,12}, however, suggest that they are effective collectors of small Aitken particles by means of diffusive deposition. Most commercial varieties of tobacco have 300 to 900 trichomes cm^{-2} on each side of mature leaves. The distribution of trichomes on cured tobacco is illustrated in Fig. 1. About 85% of tobacco trichomes have glandular heads, coated with a sticky exudate mixture of organic compounds. Because of the hydrophobic nature of this exudate it is unlikely that even soluble, ionic substances are washed off by precipitation. Thus, trichomes may retain the small atmospheric particles which are deposited on their glandular heads throughout the period of plant growth.

The thick distribution of trichomes maintains a stagnant air layer over the leaf surface with only the glandular heads of trichomes protruding. For the low air flow rates in the vicinity of trichome heads, the only effective mechanism

for airborne particle capture is likely to be diffusive deposition due to Brownian motion of the particles. The efficiency of this process varies inversely with particle size and flow rate and thus may result in the selective deposition of particles of $\sim 0.1 \mu\text{m}$ diameter and smaller. Radon daughters in air are concentrated on small Aitken particles^{13,14}. Impactor measurements (my unpublished results for radon daughter aerosol properties, with H. E. Moore and S. E. Poet) show that $\sim 90\%$ of airborne ^{210}Pb resides on particles $\leq 0.3 \mu\text{m}$ diameter at concentrations between 2×10^3 and 10^4 pCi g^{-1} . The ^{210}Pb concentration increases markedly with decreasing particle size¹⁴. Thus Brownian capture should give rise to high ^{210}Pb concentrations on tobacco trichomes.

Preliminary measurements of ^{210}Pb on cured tobacco leaves by analysis of surface particles removed by brushing and by sonic cleaning, showed ^{210}Pb concentrations of leaf surfaces up to 30 times that of the bulk leaf per g ash. Microscopy showed that trichomes were only a minor constituent of this surface debris. Subsequently, 2,000 trichomes were removed from North Carolina flue-cured tobacco and assayed for ^{210}Po by α spectroscopy¹⁵. The average ^{210}Pb activity per trichome, based on this ^{210}Po measurement and the $^{210}\text{Po} : ^{210}\text{Pb}$ ratio of the tobacco sample, was $(3.2 \pm 0.6) \times 10^{-6}$ pCi per trichome. Turkish tobacco and Kentucky burley showed $\sim 1/2$ and $\sim 1/4$ as much activity per trichome, respectively. These results indicate the accumulation and retention of tens of thousands of Aitken particles on individual trichomes, presumably on the exudate-coated glandular heads.

The solubility of separated trichomes from cured tobacco was examined. Although exudate is soluble in ether and other organic solvents before the tobacco is cured¹², the trichome heads for cured tobacco seem to be insoluble in ether, acetone and other common organic solvents as well as in concentrated HCl, HNO₃ and HCl-HNO₃ mixtures. This result is attributed to the polymerisation of exudate compounds during the curing period.

The trichomes and their glandular heads have an open, cellular structure of very low density. These properties are illustrated by the collapse of trichomes when subjected to high vacuum. ^{210}Pb activity on trichomes is probably largely associated with the exudate coatings of trichome heads. When a smoker draws on a burning cigarette, the trichomes on the tobacco fibre surfaces are subjected to temperatures from 650°C to more than 800°C ¹⁶. The pyrolysis of glandular heads of trichomes at these high temperatures should yield smoke particles with specific activities of ^{210}Pb which approach or exceed an estimated 10^6 pCi g^{-1} .

^{210}Pb in smoke particles

Published radiochemical data for inhaled mainstream smoke show an average ^{210}Po content of ~ 0.036 pCi per cigarette or ~ 2.6 pCi ^{210}Po per g smoke¹⁷, with a $^{210}\text{Pb} : ^{210}\text{Po}$ ratio of 0.66 ± 0.23^6 . The smokers' intake of ^{210}Po and ^{210}Pb within several days is equivalent to the observed excess burden of 3–10 pCi of ^{210}Pb and ^{210}Po in smokers' lungs^{6,7}. Thus most of

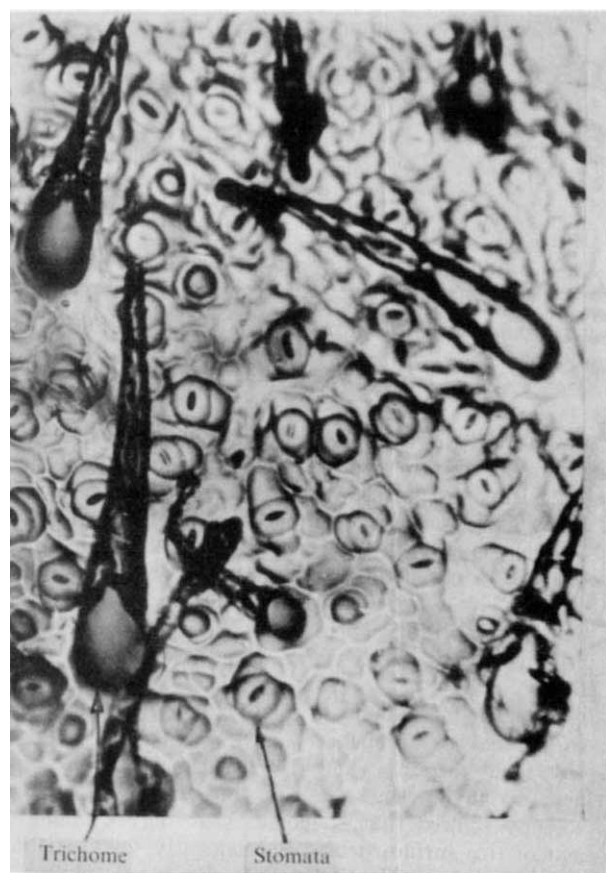


Fig. 1 Microphotograph of flue-cured tobacco, Hicks variety, bottom surface, showing trichome and stomata distribution (leaf section $550\ \mu\text{m} \times 835\ \mu\text{m}$).

the ^{210}Pb and ^{210}Po deposited in the smoker's lung must be cleared rapidly.

There is no published evidence about the physical and chemical properties of the ^{210}Pb content of insoluble smoke particles which may persist in the lung. To investigate this question, I used samples of whole condensate, prepared by standard techniques and procedures¹⁸. University of Kentucky 1R1 reference cigarettes (a non-filtered cigarette of standard US blended mixture) and Players cigarettes (all flue-cured tobacco) and large whole smoke condensate samples of each, were used.

Condensate samples were heated to drive off volatile constituents. The ^{210}Po was volatilised almost completely at temperatures between 600°C and 650°C , whereas all the ^{210}Pb remained in the residue, indicating that ^{210}Po is inhaled in volatile form and ^{210}Pb is associated with the particulate fraction of mainstream smoke.

Preliminary radiochemical results for 1R1 tobacco showed that the ^{210}Pb content was $0.40\ \text{pCi per g}$ dry tobacco, $0.81\ \text{pCi per g}$ of dry whole smoke condensate, and $130\ \text{pCi per g}$ of the non-volatile residue after slow distillation of the volatiles up to 650°C . Similarly, for Players tobacco ^{210}Pb contents were $0.48\ \text{pCi per g}$ dry tobacco, $1.46\ \text{pCi per g}$ dry whole smoke condensate, and $52\ \text{pCi per g}$ of non-volatile residue after distillation of condensate up to 600°C . The condensate residue was largely water soluble, and most of the ^{210}Pb was associated with the insoluble fraction, at a concentration of $\sim 1 \times 10^4\ \text{pCi per g}$ of residue. Preliminary microscopic examination of the non-volatile, water-insoluble residue of the condensate revealed small irregular particles, resembling combustion products, with a maximum diameter of $\sim 4\ \mu\text{m}$. Successive extractions of the residue with dilute

and concentrated HCl and HNO_3 resulted in the incomplete solution of the particles and their ^{210}Pb content. After acid extraction, translucent orange particles with the appearance of cured trichome heads were prominent in the residue.

Thus ^{210}Pb is highly enriched in the insoluble particulate fraction of inhaled mainstream smoke. Presumably the volatile smoke constituents are dispersed widely and removed rapidly from lung surfaces. Water-soluble constituents also will be quickly removed from their sites of deposition. The insoluble particles will be further fractionated during deposition and clearance, depending on their physical properties and solubility in lung fluid. The preliminary results indicate that the type of insoluble smoke particles that will persist and accumulate in the bronchi of smokers is between $\sim 10^4\ \text{pCi per g}$, the activity of water-insoluble smoke particles, and $\sim 10^5\ \text{pCi per g}$, the estimated activity of pyrolysed trichome heads.

Bronchial epithelium dose estimates

Based on the measured concentrations of ^{210}Po in epithelial samples, Little and Radford estimated a maximum dose of $\sim 200\ \text{rem per 25 yr}$ to lower-lobe bifurcations³. Holtzman's⁸ questions regarding this estimate are answered by the presence of ^{210}Pb in insoluble particles of high specific activity. The insoluble particles should, at least in part, persist sufficiently long to allow ingrowth of the ^{210}Po daughter activity. This possibility can be assessed by analysis of ^{210}Pb and ^{210}Po in small bronchial bifurcation specimens from deceased cigarette smokers.

For a single insoluble particle with $3 \times 10^{-6}\ \text{pCi } ^{210}\text{Pb}$ (the average for North Carolina flue-cured tobacco trichomes) and a $^{210}\text{Po}:^{210}\text{Pb}$ ratio of 0.8 (corresponding to particle residence time of $10^3\ \text{d}$ in the bronchi) the α radiation dose to cells within the α range (~ 6 cell diameters) would be about $0.5\ \text{rad}$ or $5\ \text{rem per year}$. However, it has been reported²⁰ that the pulmonary accumulation of insoluble dust particles involves a sequestering tissue reaction which results in high local dust concentrations in plaques or nodules. The cumulative α radiation dose to lung cells exposed continuously to high concentrations or clusters of insoluble smoke particles will substantially exceed the estimated maximum of $200\ \text{rem per 25 yr}$ based on ^{210}Po tissue measurements³. ^{210}Po and ^{210}Pb measurements of small bronchial epithelium tissue specimens from deceased smokers, as well as α autoradiography of thin tissue sections, will be needed to assess the actual α dose distribution.

The presence of insoluble particles of high specific ^{210}Pb activity in mainstream cigarette smoke should lead to their accumulation in the bronchi of smokers with residence times sufficient to allow ingrowth of the ^{210}Po daughter radioactivity. This sequence of events must explain the high local concentrations of ^{210}Po observed² in the bronchial epithelium bifurcations of smokers which indicate a maximum α dose of some $200\ \text{rem per 25 yr}$ ³. High concentrations or clusters of these insoluble smoke particles may give rise to a much higher dose in small volumes of tissue.

A significant excess incidence of lung cancer has been observed in all groups of uranium miners exposed to more than 120 working level months (WLM) of radon daughter exposure²⁰. The corresponding α radiation dose, about 0.5 to $1.0\ \text{rad per WLM}$ ²¹, is 60 to $120\ \text{rad}$, or 600 to $1,200\ \text{rem}$. Correspondingly high α radiation doses in smokers seem to be restricted to relatively small volumes of tissue, thereby suggesting a reduced risk of carcinogenesis. The results for internal emitters reported by Cember²², however, indicate that the risk for a given cumulative radiation dose may increase significantly at lower dose rates and longer periods of exposure, suggesting a higher risk for smokers.

Asbestos workers who smoke cigarettes have a very much

higher incidence of bronchial cancers than cigarette smokers²³, whereas no excess incidence of bronchial cancers was observed for asbestos workers who do not smoke. It was also reported²⁴ that insoluble dust accumulations in the bronchi contribute to lymphatic stagnation and thus to a larger accumulation of metabolic products. On this basis it is possible that the higher bronchial cancer risk among asbestos workers who are smokers may be due to the accumulation of a larger burden of insoluble ²¹⁰Pb smoke particles.

The case for insoluble α emitting particles as the primary agent of bronchial cancer among smokers is reinforced by other lines of evidence. The carcinogenicity of the chemical carcinogens in cigarette smoke has not been demonstrated for the low concentrations which occur in smoke. Insoluble radioactive particles rather than volatile constituents of smoke would seem to be the more logical agent for excess cancers in smokers on the basis of the enhanced cancer risk among smokers who inhale deeply and smoke unfiltered cigarettes, the slow decrease in the residual risk with time following cessation of cigarette smoking, and the occurrence of excess cancers in other organs of the respiratory and gastrointestinal tracts of smokers²⁵. Additionally the specific α radioactivity of insoluble smoke particles approaches that of ThO₂ which was widely used as a contrast medium in human radiography and gave rise to carcinomas of the liver and other soft tissues without involvement of chemical carcinogens²⁶.

Thus, it seems that α radiation from ²¹⁰Po in insoluble smoke particles may be the primary agent of bronchial cancer in smokers. If so, a significant risk of cancer can be attributed to chronic exposure to low levels of insoluble α emitting particles which give rise to whole organ burdens of only a few pCi of α activity, but involve very high concentrations locally in small volumes of tissue.

I thank S. E. Poet for measurements of ²¹⁰Pb and ²¹⁰Po and Art Fong and John Gardner for collecting tobacco trichomes. I also thank Ashok Taori who directed my attention to the nature and distribution of tobacco trichomes. Tobacco and condensed smoke samples were supplied by John Benner and J. H. Smiley of the University of Kentucky and J. A. Weybrew of the University of North Carolina.

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Class of promoter sites for *Escherichia coli* DNA-dependent RNA polymerase

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Some promoters for E. coli RNA polymerase contain either of two nucleotide sequences recognised by Hind endonucleases, GTCGAC or GTTAAC. Such promoters are found not only in the DNA of bacteriophages (λ and T7) but also in that of mammalian viruses, including the simian virus 40 and the human adenovirus 2.

PROMOTER sites for *Escherichia coli* DNA-dependent RNA polymerase have been defined genetically by mutations that abolish the initiation of transcription. Attempts to define

these sites biochemically have shown that, *in vitro*, strong complexes form between *E. coli* RNA polymerase and specific sites on Lac¹, T7^{2,3}, λ ⁴ and fd^{5,6} DNA. It seemed likely that these strong binding sites for RNA polymerase were the genetically defined promoter sites, although there was no direct evidence. The first such evidence came from the observation by Maniatis *et al.*⁷ that polymerase binding protects the early leftward promoter (p_L) in λ DNA from cleavage by the endonucleases prepared from *Hemophilus influenzae*. We report here results that confirm and extend these data.

The *E. coli* RNA polymerase recognises many promoters

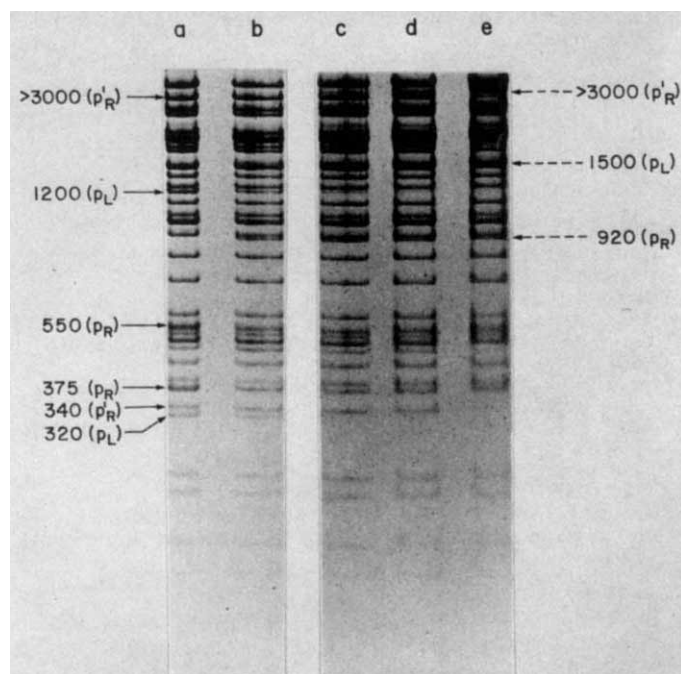


Fig. 1 Polymerase protection of Hind cleavage sites in λ DNA. RNA polymerase and λ CI857S7 DNA were prepared as described previously¹⁴. λ CI857S7 DNA (10 μ g) in 250 μ l (final volume) of 6.6 mM Tris-HCl, pH 7.5, 6.6 mM $Mg(CH_3COO)_2$, 6.6 mM β -mercaptoethanol was incubated at 37° C for 40 min with various amounts of polymerase: (a) no addition; (b) 5 μ g (molar ratio of enzyme to DNA, 30:1); (c) 10 μ g; (d) 12.5 μ g; and (e) 50 μ g. A 5 μ l sample of the Hind endonucleases¹² was added to each sample, and incubation was continued at 37° C for 12 h. The reaction mixture was chilled to 4° C and extracted with phenol, previously saturated with 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, and the aqueous phase was precipitated with two volumes of ethanol at -20° C. The samples were submitted to polyacrylamide gel electrophoresis, and the bands were stained with methylene blue as described previously¹². Fragments containing DNA sequences from the promoter sites p_L , p_R , and p_R' are shown by the solid arrows. The dashed arrows point to fused fragments, which arise as a consequence of the protection of Hind cleavage sites by RNA polymerase. The fused fragments include the indicated promoter sites, and their sizes are expressed in base pairs, as described previously¹².

in bacterial and phage DNAs, and there is evidence that it also transcribes *in vitro* the DNA from mammalian viruses such as SV40⁸ and adenovirus-2⁹. *A priori*, these facts suggest that either (1) the various promoters share a nucleotide sequence specifically recognised by the *E. coli* RNA polymerase or (2) the promoters vary in primary structure but display, in their secondary or tertiary structures, similarities which give them a specific affinity for the polymerase.

Our results suggest that several promoters, recognised by the *E. coli* RNA polymerase, are identical in at least part of their nucleotide sequence. Such promoters are found not only in the phage genomes but also in the DNA of two mammalian viruses, SV40 and adenovirus-2. These conclusions are based on the observation that the specific binding of the *E. coli* RNA polymerase protects these promoters from cleavage by an endonuclease prepared from *Haemophilus influenzae*.

The restriction endonuclease of *H. influenzae* Rd contains at least two activities, Hind II and Hind III, separable by phosphocellulose¹⁰ or DEAE chromatography and each recognising two hexanucleotide sequences. It is not known, however, whether Hind II and Hind III each contain but a single

enzyme of dual specificity or whether the fractions Hind II and Hind III themselves contain two or more different enzymes. Furthermore, *H. parainfluenzae* contains a restriction endonuclease, Hpa I, believed to recognise one of the two sequences recognised by the enzyme Hind II, as judged by its action on SV40 DNA^{10,11}. This assumption is strengthened by our observation that digestion of λ DNA with the combination Hind II/Hind III in the presence or absence of Hpa I leads to an identical pattern of cleavage products¹². Smith and Wilcox found that the Hind endonucleases recognise two palindromes of six bases on T7 DNA¹³. Because purified Hind III does not cleave T7 DNA (our unpublished result) these palindromes represent the nucleotide sequences recognised by Hind II, which are GTTAAC and GTCGAC, differing only at the two internal bases. It is not known which sequence is recognised by Hpa I.

We showed earlier that in λ DNA the early promoters p_L and p_R and the late promoter p_R' are cleaved by the Hind endonucleases, because the specific binding of *E. coli* RNA polymerase resulted in the fusion of pairs of fragments which flank these sites. In addition, for two mutants with altered p_L , $sex1$ and $sex3$, cleavage of the promoter was abolished even in the absence of polymerase. However, a mutant with a defect in p_R , $\alpha13$, did not abolish the corresponding Hind cleavage site in the absence of polymerase. We therefore concluded that the Hind sextuplet was only part of the nucleotide sequence responsible for polymerase recognition. Finally, it was suggested that p_L , p_R , and p_R' contain one of the two sequences recognised by Hind II, that sequence which is not cleaved by Hpa I.

When increasing amounts of RNA polymerase were added to λ DNA the protection from Hind cleavage proceeded in the order p_R , p_L , p_R' (Fig. 1). Protection by polymerase still occurred in the presence of ATP or the mixture ATP-GTP (Fig. 2), when the enzyme was believed to form an initiation complex at the promoter site. This supports the view that the strong binding sites for polymerase described here coincide with, or at least overlap, the promoter sites. It does not agree with the suggestion¹⁵ that the RNA polymerase binds far from the initiation site of transcription. By contrast, if all four triphosphates were included in the reaction mixture, the protection was abolished, presumably because the polymerase moves away from the promoter site as it transcribes the DNA. The RNA synthesised was heterogeneous in size, and superimposes a diffuse pattern in the lower part of the gel (Fig. 2). In our experiments, the triphosphates were added after the formation of the polymerase-promoter complexes. If rifampicin, an inhibitor of polymerase binding to the first purine nucleoside triphosphate¹⁶, was added together with the triphosphates, the pattern of protection by polymerase was not altered (Fig. 2).

Other promoter sites in λ DNA¹⁴ (for example, in *b2* and in the *O-P* region, or p_c and p_c' that control transcription of gene *cI*) failed to protect a Hind cleavage site by binding of *E. coli* RNA polymerase. This raises the possibilities that either (1) these promoters bind the polymerase but do not contain the nucleotide sequence recognised by Hind, or (2) these promoters include a Hind II sextuplet but binding of polymerase is not detected in the test situation used. These possibilities have not been investigated.

Promoter sites including a Hind sextuplet were also detected in T7 DNA. Figure 3 shows that when T7 DNA was preincubated with increasing amounts of *E. coli* RNA polymerase (enzyme:DNA molar ratios from 30 to 250), four sites were successively protected from cleavage by the Hind endonucleases. Under the same conditions, but with Hpa I replacing the Hind endonucleases, two sites were protected, but only at the higher polymerase:DNA ratios. The protec-

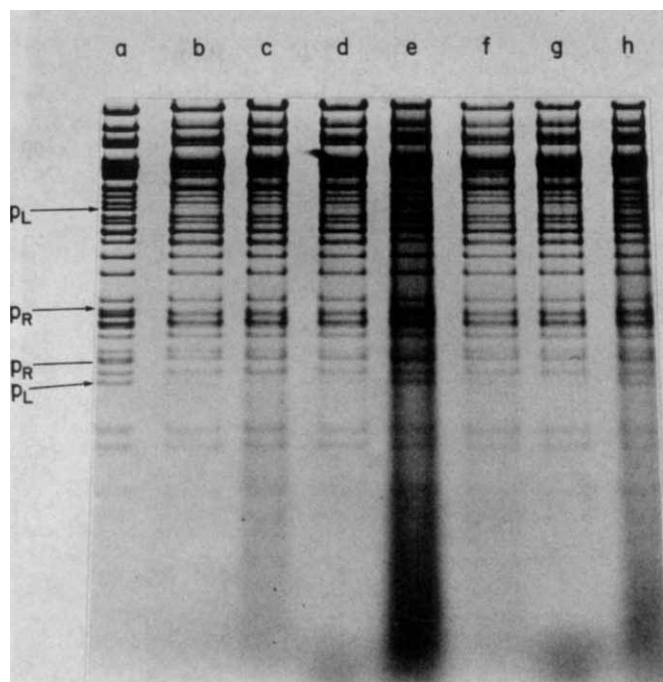


Fig. 2 Effect of ribonucleoside triphosphates on the polymerase protection. λ CI857S7 DNA (10 μ g) was incubated with RNA polymerase (20 μ g) under the conditions shown in the legend to Fig. 1. (Sample (a) is a control without polymerase.) Then various combinations of ribonucleoside triphosphates were added as follows: (a and b) no addition; (c) 60 μ g of ATP; (d) 60 μ g each of ATP and GTP; (e) 60 μ g each of ATP, GTP, CTP, and UTP; (f) 60 μ g each of ATP and GTP, and 2 μ g of rifampicin; (h) 60 μ g each of ATP, GTP, CTP, and UTP, and 2 μ g of rifampicin. After incubation at 37° C for 10 min, 5 μ l of the Hind endonucleases was added to each sample and incubation was continued at 37° C for 12 h. Extraction with phenol and analysis by polyacrylamide gel electrophoresis were as described in Fig. 1. Fragments containing DNA sequences from the promoter sites p_L , p_R , and p_R' are shown by the solid arrows. The dashed arrows point to fused fragments, which arise as a consequence of the protection of Hind cleavage sites by RNA polymerase.

tion resulted in the appearance of two bands (Fig. 3) missing when preincubation with polymerase was omitted. It seems therefore that in T7 DNA two strong promoters contain the sequence found in p_L , p_R and p_R' in λ DNA, that is, the sequence recognised by Hind II but not by Hpa I. Two weaker promoters were cleaved by Hpa I and differed from the other two. However, because the sextuplets recognised by Hind II differ only by two internal base pairs, as noted above, the promoter sites cleaved by Hind II alone, or cleaved by both Hind II and Hpa I, are closely related in at least part of their nucleotide sequence.

The DNAs from two mammalian viruses, simian virus 40 (SV40) and adenovirus-2 (Ad2) stimulate *in vitro* transcription by the *E. coli* RNA polymerase^{8,9}. Interestingly, in each of these viral DNAs, binding of the *E. coli* polymerase prevented cleavage by Hind at one site. In SV40 DNA the Hind cleavage site between fragments B and G (Fig. 5A) was protected, at position 18% on the standard map (Fig. 4). In fact, this site was cleaved by Hpa I, and polymerase binding protected one site from cleavage by this endonuclease (Fig. 5A). It seems therefore that a promoter for *E. coli* polymerase in SV40 was cleaved by Hpa I, as were two promoters in T7 DNA. Dhar *et al.*¹⁷ have shown that *in vitro* transcription of SV40 DNA is initiated in this region of the SV40 genome and proceeds anticlockwise through the G fragment. Their

data show that the G fragment, which is about 350 base pairs long, is cleaved by the restriction endonuclease RII into two fragments. The smaller of these represents approximately one-third of the Hind G fragment, that is, 120 base pairs, and contains the first sequences transcribed. Extensive sequence analysis of this transcript¹⁷ revealed that the recognition site for the RII endonuclease occurs at position 115 from the start of the transcript. This implies that the promoter is extremely close to the Hind recognition site and also close to the start of transcription, in agreement with the results obtained for λ DNA (Fig. 2). Figure 5B shows that Ad2 DNA also contains a Hind cleavage site that can be protected by *E. coli* RNA polymerase. The protection was not observed with Hpa I. It is tempting therefore to speculate that this site is a promoter for *E. coli* RNA polymerase *in vitro*, of the same type as the λ and T7 promoters. An estimate of its location in Ad2 DNA can be deduced from the cleavage pattern of the hybrid Ad2-SV40 (Ad2⁺ ND1) DNA. Ad2⁺ ND1 derives from Ad2 by two alterations in the viral DNA. (1) A portion of Ad2 DNA has been deleted between the positions 14.0% and 19.4%, measured from one end of the DNA molecule¹⁸. (2) Instead, a portion of SV40 DNA has been inserted, covering the region between 11% and 28% of the standard SV40 map. Interestingly, Ad2⁺ ND1 contained the Hpa I cleavage site between SV40 DNA fragments B and G, and Fig. 5C shows that two fragments, missing from Ad2 DNA, were fused in the hybrid as a consequence of the polymerase protection. However, the polymerase failed to protect any other Hind cleavage site in the hybrid. Thus it can be deduced that the polymerase binding site that was detected in Ad2 DNA must be in the part of the molecule that is deleted in the hybrid, that is, somewhere between map positions 14.0% and 19.4%.

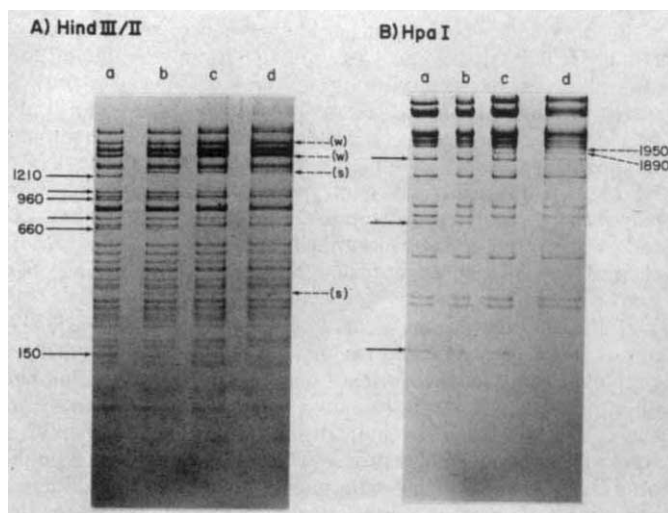


Fig. 3 Polymerase protection of Hind and Hpa I cleavage sites in T7 DNA. T7 DNA (8 μ g) was incubated with varying amounts of RNA polymerase as shown in the legend to Fig. 1. (a) no addition; (b) 5 μ g; (c) 12.5 μ g, and (d) 40 μ g. Then 5 μ l of the Hind endonucleases (A) or 10 μ l of purified Hpa I (gift of J. Sambrook¹¹) (B) was added and the incubation was continued at 37° C for 12 h. The samples were prepared and analysed as described in Fig. 1. The dashed arrows point to fused fragments which arise from the protection of cleavage sites. The letters in parentheses indicate whether the fused fragments contain a weak (w) or a strong (s) promoter site, as judged by the amount of polymerase needed to detect their appearance. The solid arrows point to fragments which progressively disappear when increasing amounts of polymerase are tested. The sizes of the fragments are expressed in base pairs (Fig. 1).

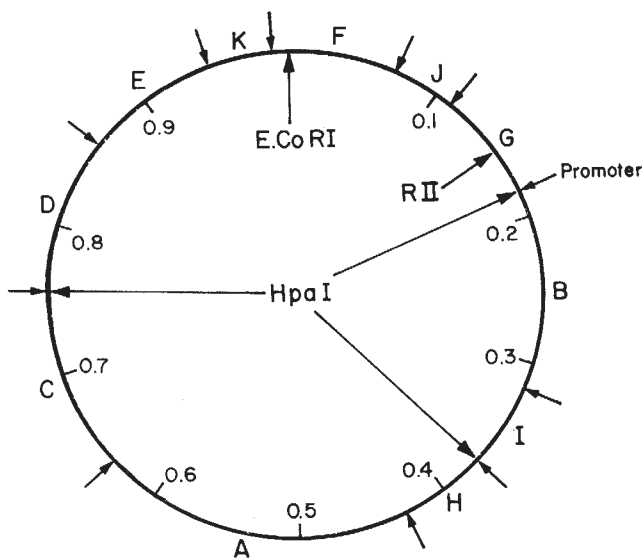


FIG. 4 Physical map of fragments in SV40 DNA. The map was adapted from Danna *et al.*¹⁰ The arrows outside the circle indicate the sites of cleavage by the Hind endonucleases, and the resulting fragments are designated by letters (A to K)¹⁰. The sites of cleavage by Eco RI, Hpa I, and the site of cleavage by RII within the Hind fragment G are indicated by arrows inside the circle.

Our results support the conclusion that a class of promoters for *E. coli* RNA polymerase shares identical nucleotide sequences, at least those contained in the specific sextuplets recognised by Hind II. The strongest evidence that a part of the Hind sextuplets actually belongs to the promoter sites comes from the fact that two mutations that altered a genetically defined promoter in λ DNA, *sex1* and *sex3*, also altered the nucleotide sequence recognised by Hind II. Clearly, sequences outside these sextuplets are also involved in specifying promoter sites, because only a small proportion of the Hind II cleavage sites can bind polymerase. The lengths of these additional sequences are unknown, but they presumably could not exceed the 47 to 52 base pairs protected from pancreatic DNase digestion when *E. coli* polymerase binds to promoters³. To what extent the promoter sites described here are homogeneous in their DNA sequences outside the Hind II sextuplets has yet to be determined.

It must be noted that, for λ and T7 DNAs at least, the number of RNA polymerase molecules (30–250) needed to protect Hind cleavage sites exceeded by far the number of promoters or strong binding sites (7–10) reported in these DNAs^{15,19}. This indicates that either (1) only a fraction of the molecules in the polymerase preparation could bind DNA; or (2) the experiments were performed under conditions of kinetic control and reflect differences in the rate of complex formation; or (3) the experiments were performed under conditions of thermodynamic control, equilibrium was attained, and the differences reflect differential affinities; or (4) several polymerases were bound at single sites. In the latter case, however, the multiple polymerase molecules would probably not have been arrayed linearly, because polymerase binding to promoter sites only protects stretches of 47–52 nucleotide base pairs from digestion by pancreatic DNase³. Experiments are in progress to decide among these possibilities.

As we pointed out earlier, several promoter sites are not identified by polymerase protection of a Hind II site. Such promoters may not contain any of the Hind II sextuplets. Alternatively, they may include one of them but not bind the polymerase to a detectable extent under the conditions

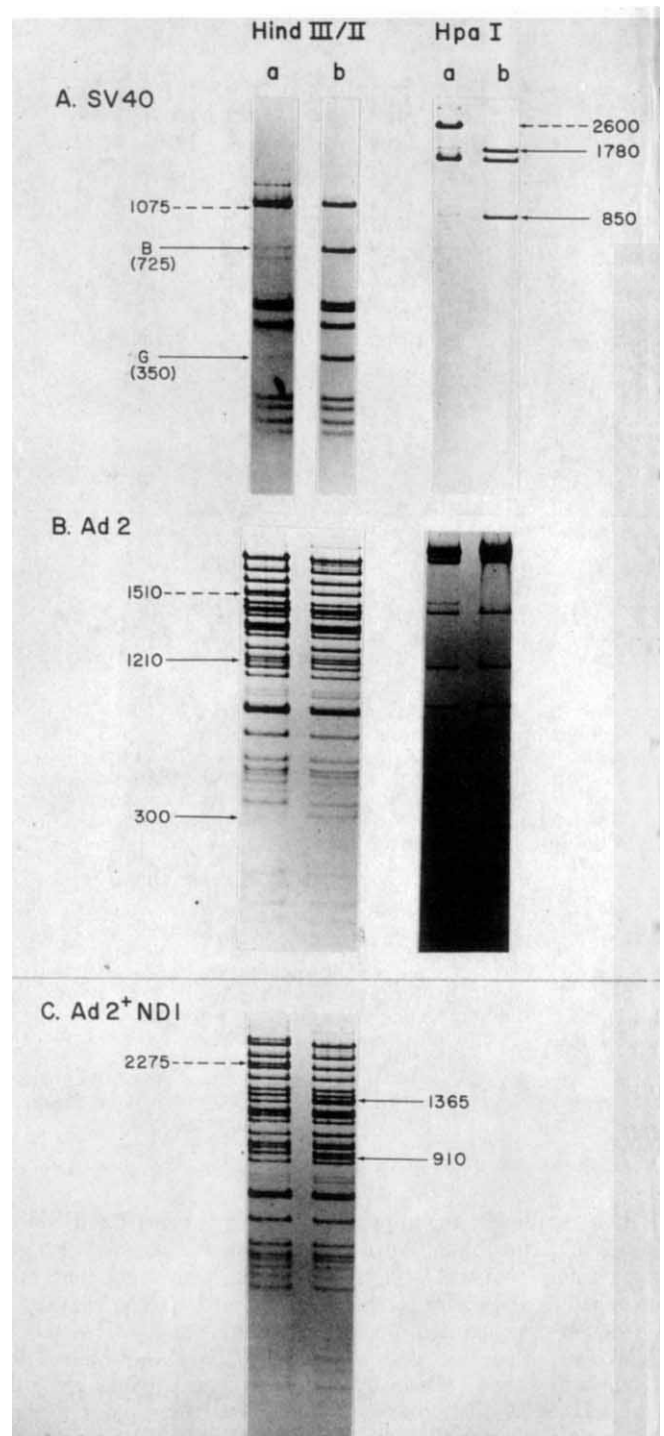


FIG. 5 Polymerase protection of sites cleaved by Hind III/II and Hpa I endonucleases in SV40, Ad2, and Ad2⁺ ND1 DNAs. SV40 (A), Ad2 (B), and Ad2⁺ ND1 (C) DNAs (10 μ g) were incubated in the presence (a) or absence (b) of *E. coli* RNA polymerase (20 μ g) at 37° C for 40 min as described in the legend to Fig. 1. Then 5 μ l of Hind III/II was added and the incubation was continued at 37° C for 12 h. Subsequent extraction and analysis were performed as described in the legend to Fig. 1. In the case of samples subsequently digested with Hpa I (10 μ l), 5 μ g of each DNA was used with 20 μ g of polymerase. The solid arrows indicate fragments that are present when polymerase is omitted from the reaction mixture. The dashed arrows indicate fused fragments arising as a result of polymerase protection. The sizes of the fragments are expressed in base pairs. In the Hind III/II pattern of SV40, the positions of fragments B and G (Fig. 4) are indicated.

used. The first hypothesis was verified in one case at least, that of the wild type promoter for the *lac* operon. This conclusion arose from the mapping data of the Hind II-III fragments generated from *lac* DNA in the well characterised λ plac5 DNA²⁰ (in collaboration with P. Tiollais, unpublished data). The mapping data indicate that there is no Hind cleavage site within the first 500 base pairs in the *lac* DNA, which, in this insertion, extends from the promoter (*p*) through the operator (*o*) to the end of the gene for β -galactosidase (*z*). This is a clear indication that *p* is not cleaved. Thus it seems that the promoter sites analysed in this report represent a subset only of the promoters recognised by *E. coli* RNA polymerase.

The fact that we could identify a strong promoter site for the *E. coli* polymerase in the DNAs from two mammalian viruses, SV40 and Ad2, is intriguing. Clearly, a random base distribution would not be likely to generate a sequence of this presumed complexity in DNA molecules as small as 26 million daltons (Ad2) or 3 million daltons (SV40). Thus it is tempting to believe that these sequences are of biological importance for SV40 and Ad2. In SV40 DNA, the early RNA molecules are synthesised *in vivo* from a promoter located at a site widely distant from the promoter site recognised by the *E. coli* polymerase. It seems therefore that in the early stage of the viral development the mammalian polymerase does not recognise the same promoter site as is recognised by the *E. coli* enzyme. Whether the mammalian enzyme recognises this site at some later stage is unknown. It would be of particular interest to carry out similar experiments with the mammalian polymerase.

Since this report was submitted for publication, Drs S. Weissman and K. Murray, who independently communicated this result to us, have shown that Hpa I recognises the sequence GTTAAC. This means that the sequence GTCGAC is associated with the strong binding sites for *E. coli* RNA polymerase, and the sequence GTTAAC is associated with the weaker binding sites. Furthermore, from the recent sequence analysis communicated by Dr Weissman it is now known that the starting site for transcription in fragment G of SV40 is located 29 base pairs from the Hpa I sextuplets which delimits fragments B and G.

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Promoters are in the operators in phage lambda

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E. coli RNA polymerase binds to a specific site within each λ operator. This DNA site is recognised by three proteins: RNA polymerase, the λ phage repressor, and a restriction endonuclease (Hin).

EACH of the two operators in bacteriophage λ , O_L and O_R , consists of catenated, nonidentical repressor binding sites¹⁻³ (Fig. 1). At each operator, repressor first binds to a terminal site, and subsequent molecules add in the direction opposite to that of transcription of the adjacent controlled operons. Moreover, although the two operators are not identical, each contains, between the first and second repressor binding sites, the same short sequence that is cut by a restriction endo-

nuclease (Hin) isolated from *Haemophilus influenzae*. We show here that both operators contain a specific binding site for RNA polymerase (promoter). This operator-promoter site includes the restriction endonuclease target, and we conclude that repressor, RNA polymerase, and Hin recognise shared DNA sequences. A preliminary account of related experiments was presented elsewhere³. Experiments similar to some of those recorded here and in ref. 3 are also reported in the accompanying article by Allet *et al.*⁴

Promoter mutations in the operators

A mutation has been described, called *sex1*⁵ that decreases transcription *in vivo* and *in vitro* of the operon controlled by

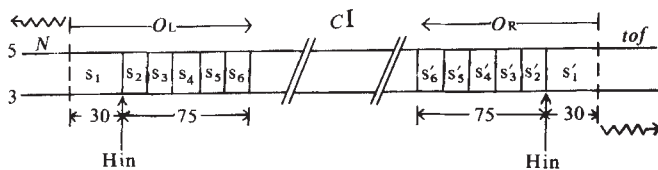


Fig. 1 Schematic representation of the λ operators and adjacent genes. The wavy arrows show the direction of transcription of the repressor-controlled genes *N* and *tof*. Two arrows indicate the order of repressor binding from the first sites (s_1 and s'_1) in O_L and O_R . The *Hin* cutting sites in O_L and O_R are indicated along with the number of base pairs within the operators on either side of each *Hin* cut. The repressor gene is *cI*.

O_L ^{6,7}. We have shown³ by RNA polymerase-DNA binding experiments that *sex1* decreases the affinity of a DNA fragment bearing the leftward promoter, P_L , for RNA polymerase, and we concluded therefore that the mutation is in that promoter. We now wish to ask, where is *sex1*, and hence the promoter, located in relation to O_L ?

The experiment of Fig. 2 shows that *sex1* is located within O_L , in the sequence recognised by *Hin*. We compared a *Hin* digest of whole λ wild-type DNA with that of λ *sex1* DNA (a and b). The figure shows that the fragments designated *Hin* 320 and *Hin* 1125, generated from λ wild-type DNA, are replaced by a larger fragment about 1,450 base pairs long in the digest of λ *sex1* DNA. This is the only difference in the two gel patterns. To show that this difference is attributable to the *sex1* mutation itself, and not to some irrelevant difference between λ wild-type and λ *sex1* DNA, we isolated revertants with wild-type plaque morphology from λ *sex1*, as described in the legend to Fig. 2. The figure (c and d) shows *Hin* digests of examples of the two classes of revertants recovered. In one (d) the original *Hin* cutting site is restored, and in the other this site remains defective. (Three out of five revertants tested, not necessarily representing independent back mutations, lack the original *Hin* site.) We have shown previously^{2,3} that the junction between *Hin* 320 and *Hin* 1125 is the *Hin* cutting site in O_L . We conclude, therefore, that the *sex1* mutation alters the sequence of the O_L *Hin* site. Allet and Solem⁸ have reported, and we have confirmed (unpublished), that *sex3*, an independently derived leftward promoter mutation (personal communication from M. Gottesman), also alters the O_L *Hin* site.

We have also found that DNA bearing a rightward promoter mutation, $x3^9$, lacks the O_R *Hin* site (not shown), in formal analogy to the alteration of the O_L *Hin* site by *sex1*. Although we have not studied x^+ revertants, this finding is consistent with other evidence, presented below, that the rightward promoter includes the O_R *Hin* site. (It should be noted that not all so-called x mutations alter the O_R *Hin* site; $x13$, for example, located to the right of $x3^9$, does not affect this site⁸. As suggested by Ordal and Kaiser⁹, who presented evidence for interpenetration of operator and promoter at O_R , the mutation $x13$ may block rightward transcription by action distal to the promoter.)

Binding of RNA polymerase to *Hin* sites

The experiment of Fig. 3 shows that RNA polymerase binds with special avidity to the two *Hin* sites located in O_L and O_R . To perform this experiment we digested whole ³²P-labelled λ DNA with *Hin* in the presence of varying amounts of RNA polymerase. Most of the *Hin* cutting sites were unaffected

by the presence of polymerase, but at the lowest ratios of polymerase to DNA used two sites were specifically protected. These two sites are those previously mapped in O_L

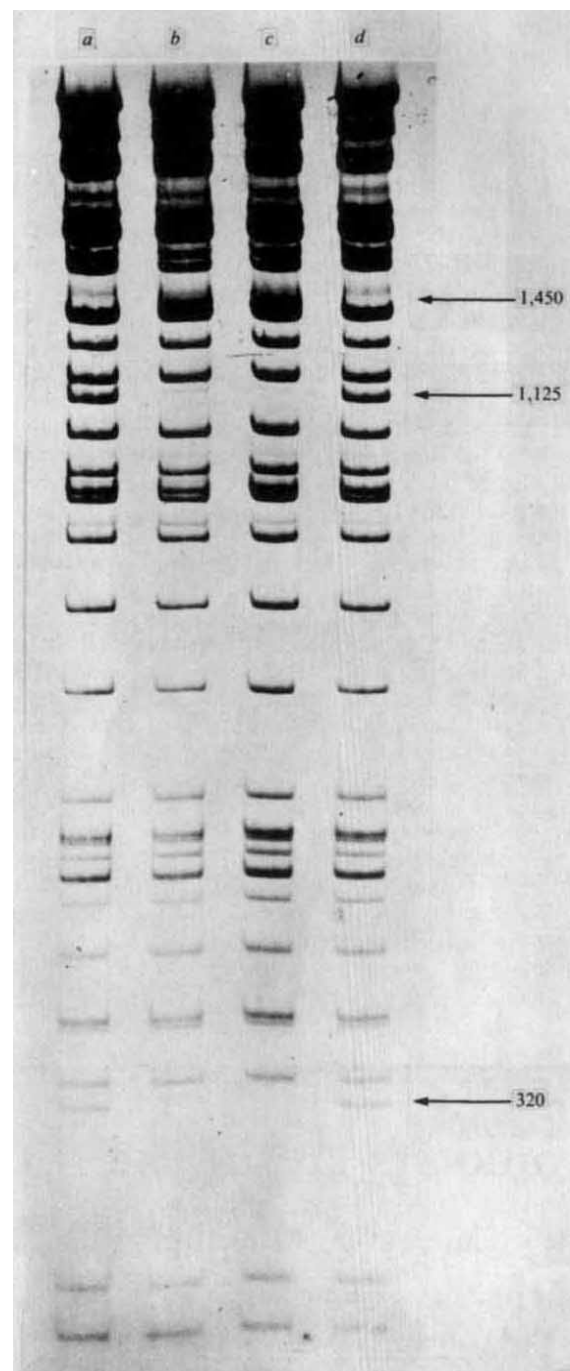


Fig. 2 *Hin* digestion patterns of DNA from λ , λ *sex1* and two λ *sex1* revertants of λ *sex1*. DNA isolated from each phage (100 μ g) was digested with *Hin* as described in ref. 3 except that no additional carrier DNA was added. *Hin* was isolated from the exonuclease-free strain Rd com⁻¹⁰ (ref. 13) as described in ref. 3. The digested DNA was fractionated by electrophoresis in a 3.5% TBE gel¹, and visualised by staining with 0.05% methylene blue. *Sex1* revertants of λ cI857*sex1*S7 were isolated by picking large plaques on an *suIII*⁺ strain, which is permissive for the growth of *S7* phages. An *su*⁻ strain was then lysogenised separately with these revertants, and high titre stocks were prepared by thermal induction. a, λ Wild type; b, λ *sex1*; c, λ *sex1* revertant lacking the O_L *Hin* site; d, λ *sex1* revertant bearing a restored O_L *Hin* site. The labels in the figure give the number of base pairs in the indicated fragments, as determined in ref. 3.

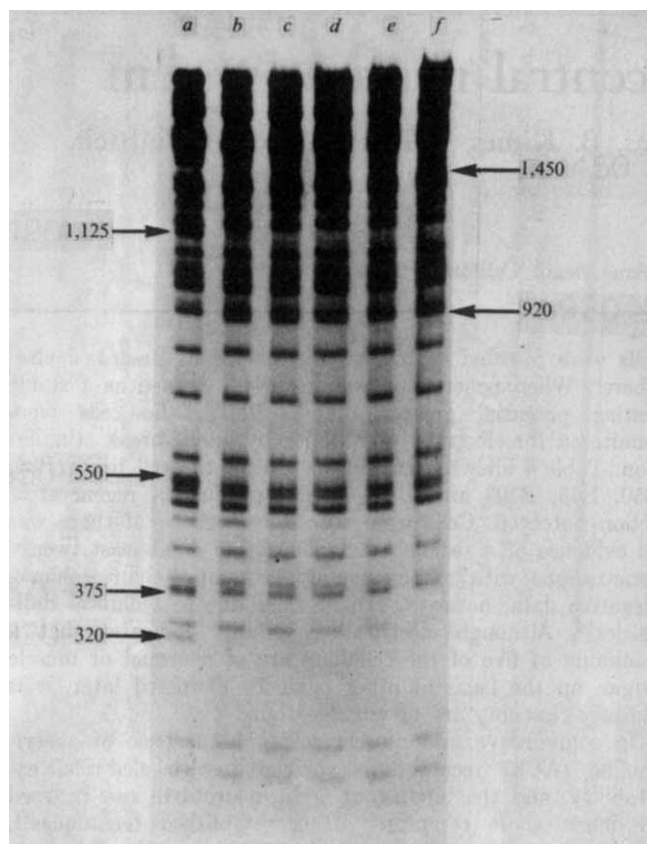


FIG. 3 Autoradiogram of λ DNA digested with Hin in the presence of various amounts of RNA polymerase. 3 μ g of 32 P-labelled λ DNA (about 10^5 c.p.m. μ g $^{-1}$) was incubated with RNA polymerase for 5 min at 37°C in a solution containing 50 mM KCl, 8 mM MgCl₂, 20 mM Tris, pH 8.0, 0.1 mM DTT, 5% DMSO and 0.1 mM each rATP and rGTP. Then 50 μ l of Hin buffer² was added with Hin, and the mixture was incubated for another 60 min at 37°C. After phenol extraction the products were examined as in Fig. 2. a, No RNA polymerase (RNP); b, 1.5 μ g RNP; c, 3 μ g RNP; d, 4.5 μ g RNP; e, 6 μ g RNP; f, 7.5 μ g RNP.

and O_R . Thus Hin 320 and Hin 1125, the junction of which is in O_L , were replaced by a larger fragment, and, similarly, Hin 375 and Hin 550, the junction of which is in O_R , were replaced by a larger fragment. Figure 3 also shows that at ratios of RNA polymerase to DNA higher than that required to protect the Hin sites in O_L and O_R , two additional sites were protected. Allet and Solem⁸ suggest a location for one of these additional sites, but the location of the fourth site is as yet unknown.

The RNA polymerase protection of the O_L Hin site in whole λ DNA is consistent with data presented elsewhere showing a similar effect of polymerase on Hin digestion of a DNA fragment bearing an intact O_L ³. However, the protection of the O_R Hin site, also observed in the experiment of Fig. 3, contradicts our earlier unexplained failure to observe protection of that site when the experiment was performed with a specific fragment bearing an intact O_R . In recent experiments with this fragment we have observed protection of the Hin site in O_R at ratios of polymerase to DNA comparable with those used in the experiments of Fig. 3 (unpublished).

Relationship of promoter to operator

The results of our experiments show that at both O_L and O_R , repressor and RNA polymerase recognise in common a

specific DNA base sequence which includes a Hin restriction endonuclease cutting site. Since the Hin site occupies a corresponding position in each operator, the relationship of promoter to operator is apparently the same at O_L and O_R . These findings confirm a specific mechanism of repression at the λ operators: repressor bound to the operator prevents binding of RNA polymerase to the promoter. It is a remarkable fact that mutations in the operators can affect RNA polymerase binding without, apparently¹⁰, affecting repressor binding.

Our results also require a reconsideration of the hypothesis of RNA polymerase 'drift'¹¹ between promoter and transcription start point. From the following facts we deduce that the O_L Hin site (and hence the RNA polymerase binding site) is within 30–50 base pairs of the start point of transcription of gene *N*. First, there are 30 base pairs between the O_L Hin site and the left terminus of O_L ², and second, the left terminus of O_L is within 20 base pairs of the start point of transcription³. Our deduction is proved directly by DNA sequence analysis which reveals that 33 base pairs separate the O_L Hin site from the start point of transcription (Maniatis *et al.*, in preparation). We do not yet know precisely how many bases on each side of the Hin cutting site are covered by bound RNA polymerase, but since DNA-bound RNA polymerase covers about 40 base pairs¹², it is possible that polymerase bound at the O_L Hin site extends to the transcription start point. In any case it is clear that RNA polymerase 'drift', if it occurs at all, is certainly over a much shorter distance than the 100–300 base pairs previously estimated¹¹.

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Note added in proof: A slightly modified interpretation of our findings, which we cannot yet exclude, is that RNA polymerase binds adjacent to (that is within a few bases of) the Hin site rather than directly over it. This scenario would require a more complicated explanation of the effects of the *sex* mutation than the one we have suggested.

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Clonal cell lines from the rat central nervous system

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Five neuronal and a large collection of putative glial cell lines from the rat central nervous system have been established in clonal cell culture and partially characterised. These cells shed new light on the distribution of neurotransmitter synthesis and brain-specific antigens among nerve and glia.

ALTHOUGH the C1300 mouse neuroblastoma^{1,2} has been useful for studies of the differentiation and trophic interactions of nerve cells, many more neuronal cell lines will be required before generalisations can be made about the behaviour of these cells in culture and maximum use made of the clonal culture system. This also applies to the glia and other satellite cells, whose role in the metabolism and physiology of nervous tissue is poorly understood; only a few clonal cell lines of glial origin have been described³⁻⁵.

To establish defined cell lines from the central nervous system (CNS), cells must first be adapted to permanent culture and cloned; at present neoplasms are the only efficient source of dividing cells for this purpose. The established clones must then be sorted into cell types, for example, neurones must be distinguished from glia, and, among the glial cells, astrocytes from oligodendrocytes. We describe here an attempt to establish and characterise several clonal cell lines from the rat CNS. Of approximately 120 cell lines isolated from independently arising tumours so far, some properties of 22 clones from independently arising tumours are described.

Establishment of cell lines

Neoplasms were induced transplacentally with nitrosoethylurea (NEU)⁶. BDIX rats were injected 15 d after conception with 4.0 mg of NEU per 100 g body weight, which reduced the litter size by 50%. Between 4 and 10 months after birth, approximately half the offspring had symptoms of extreme nervous system disorders and were examined for tumours. In 93% of the animals, tumours were found in the CNS; the others had tumours in other areas. Excised tumours were minced finely in modified Eagle's medium containing 20% foetal calf serum⁷, cells (including pieces of tissue) were diluted and 1×10^4 – 1×10^6 cells were plated on Falcon 60 mm tissue culture dishes. Each dish was examined periodically for cell proliferation, and as areas of extensive growth were found, cells were isolated by cloning rings and transferred to another dish. Since the initial explants contained cells of widely different morphologies, the most complex cell types were selected. When these cells reached confluency, they were either cloned or passaged for further study. Only one clonal cell line was initially obtained from each tumour. Of the fourteen cell lines examined, all were near diploid.

Excitability and morphology

Mammalian nerve or muscle can be distinguished from glia and most other cells, as the former have electrically excitable membranes⁸. To assay for membrane excitability,

cells were impaled with a microelectrode as described elsewhere⁹. When penetration was achieved, defined as a stable resting potential greater than -30 mV, the cells were monitored for electrical excitability by anode-break stimulation. Table 1 shows that at least five of the cell lines (B35, B50, B65, B103 and B104) could produce a regenerative action potential. Cells were scored as negative if there was no evidence of a regenerative response after at least twenty penetrations with resting potentials greater than -30 mV. Negative data, however, can be false due to technical difficulties¹⁰. Although electrical excitability indicates that a minimum of five of the cell lines are of neuronal or muscle origin, on the basis of other evidence discussed later, it is unlikely that any are of muscle origin.

In some nerve and muscle cells the presence of acetylcholine (ACh) receptors is correlated with electrical excitability, and the binding of 125 I- α -neurotoxin can be used to detect these receptors. Using established techniques¹¹, the number of curare-protectable α -neurotoxin-binding sites was determined in several cell lines (Table 1). Of the twelve new lines examined, three cell lines with excitable membranes have detectable ACh receptors. Clones of the C1300 neuroblastoma and the L6 myoblast line also bind toxin, while 3T3 fibroblasts do not.

After cloning, the cells could be classified into six morphological groups (Table 1 and Fig. 1). Four of the five neuronal lines—B35, B50, B65 and B103—fall into a class with clones of C1300 neuroblastoma (group 2); they all

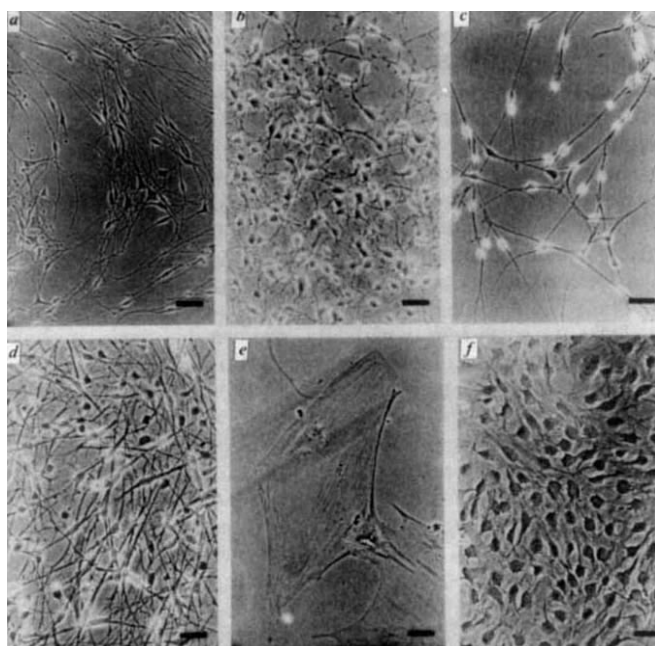


Fig. 1 Phase contrast photomicrographs of representative cell lines. The bar in each photograph represents 50 μ m. a, B6 (group 1); b, B35 (group 2); c, B103 (group 2); d, B111 (group 3); e, B25 (group 5); f, B12 (group 6).

TABLE 1 Characteristics of cell lines

Line	Group	Excitable membrane	α -Neurotoxin	O-Serum	DBcAMP	ChA	TH	GAD	GABA	AChE	BChE	AChE/BChE	%HP	S100	14-3-2
B1	IV	—	—	—	—	16				<1	<1	—	4.5	<5	<50
B6	I	—	—	—	++	31			6.3	16	1	16	4.1	21	230
B9	III	—	—	—	+++	30								16	780
B11	VI	—	—	—	++	15		<20	13	1,200	880	1.4	2.0	49	560
B12	VI	—	—	—	+	12	7		2.1	540	380	1.4		32	930
B15	I	—	<1	—	++	41		<20	1.5	100	60	1.7		66	<50
B19	I	—	—	—	±	5	19			51	15	3.4		42	330
B23	VI	—	<1	—	+++	36	7	<20	5.6	1,200	860	1.4	2.6	15	
B25	V	—	—	—	±										
B27	I	—	—	—	±	33				540	320	1.7	4.7	<5	80
B35	II	+	<1	++	++	40	<2	330	7.1	100	60	1.7	0.7	<5	520
B49	IV	—	<1	—	+	<4	<2	<20		1,200	830	1.4	8.2	24	500
B50	II	+	<1	++	++	<4	7	1,350	30	2,300	1,800	1.3	1.8	45	560
B56	I	—	—	—	±	<4				38	38	1	2.3	<5	<50
B65	II	+	5.4	++	++	25*	50	1,200	84	130	90	1.4	1.5	28	610
B82	II	—	<1	—	+++	24	11	<20	1.1	310	280	1.1	1.8	86	3,300
B90	V	—	<1	—	++	38	10	<20	1.4	70	70	1		20	
B92	III	—	<1	—	++	<4	6		2.1	5,300	4,300	1.2	3.6	21	1,120
B103	II	+	7.9	+++	+++	33	<2	1,180	29	270	130	2.1	2.2	10	450
B104	I	+	8.2	+++	+++	77	6	<20	6.3	160	50	3.2	4.2	17	570
B108	VI	—	<1	—	+++	<4	22	<20	3.4	3	3	1	0.7	20	1,250
B111	III	—	—	—	—	<4	8	<20		290	190	1.5	2.6	33	450
C1A	II	+	5	+++	+	15			4.2	1,100	15	76	1.4	<5	500
S20	II	+	1			400			9.4						<50
L6†	IV	+	400	—	—	10	<2	<20	<0.1	12	5	2.4	4.9	<5	<50
Brain						500	450	6,000						350	890
3T3	IV		<1	—	+	<4	<2	<20	<0.1	17	3	5.7	18	<5	<50
Liver						<4									<50
P3				—	—	<4			<0.1	4	4	1	<0.01		

With noted exceptions, stationary phase cells from passages 5 to 10 after cloning were assayed for enzyme activities, proteins and morphologies as described in the text. The amount of S100 and 14-3-2 is expressed as ng specific protein per 100 ng of total soluble protein. Bovine antigens were used in both cases; these cross react extensively with the respective rat proteins (H. Herschman, personal communication). The enzyme-specific activities are expressed as follows: esterase, using either acetylthiocholine or butyrylthiocholine as substrates, ΔA_{412} per min per mg protein; choline acetyltransferase, pmol ACh formed per min per mg protein; tyrosine hydroxylase, pmol $^{14}\text{CO}_2$ released per 20 min per mg protein; glutamic acid decarboxylase, pmol $^{14}\text{CO}_2$ released per 20 min per mg protein inhibitable by 1 mM aminooxyacetic acid. The neurotransmitter products were identified chromatographically. GABA assays were done on trichloroacetic acid soluble cell supernatants using an amino acid analyser with expanded resolution (D.S. and W.C., in preparation). The results are expressed as residues of GABA per 1,000 residues of free amino acids. The effects of serum-free medium and 1 mM dibutyryl cyclic AMP on cell morphology were assayed on exponentially dividing cells and range from no change (—) to the greatest change (+++); an effect greater than (+) is considerable. The percentage hydroxyproline is expressed as the percentage of proline found in secreted protein which is hydroxylated (see ref. 33 for discussion and assay procedures). The binding of ^{125}I - α -neurotoxin is expressed in 10^{-15} mol toxin bound per mg protein. The morphological groups indicate the general morphologies of the cells (see Fig. 1 and text). Electric excitability is scored as positive if regenerative action potentials are observed, and negative if there was no evidence for this event in at least 20 cells examined with resting potentials more negative than -30mV . C1A and S20 were clones of the C1300 mouse neuroblastoma^{12,34}. L6 was a clonal rat myoblast line³⁵, 3T3 was a fibroblast cell line, and P3 an immunoglobulin-secreting plasmacytoma. O-serum, the effect of serum-free medium on cell morphology; DBcAMP, the effect of dibutyryl cyclic AMP on cell morphology; ChA, choline acetyltransferase activity; TH, tyrosine hydroxylase activity; GAD, glutamic acid decarboxylase activity; GABA, residues of GABA per 1000 residues of free intracellular amino acids; AChE, esterase activity using acetylthiocholine as substrate; BChE, esterase activity using butyrylthiocholine as substrate; AChE/BChE, the numerical ratio of the two esterase activities; % HP, percentage of the total secreted proline found as 4-hydroxyproline; S100 and 14-3-2, concentrations of these proteins in cell lysates.

* Exponentially growing cells.

† All assays were done in fused, multinucleate myotubes, except for the effects of dibutyryl cyclic AMP and O-serum medium on cell morphology. Myoblasts are of group 4 morphology.

grow loosely associated with the surface of the culture dish and have relatively spherical, phase-bright cell bodies. A fifth neuronal cell line, B104, has a group 1 morphology, with very long, thin and usually bipolar cells. The other groups consist of cells with short, highly branched processes (group 6), extremely large flat cells (group 5), cells which are large and flat during the exponential phase of growth, but which branch profusely in the stationary phase (group 3), and the fibroblast-like cell (group 4). Whenever more than one cellular morphology was observed during the growth cycle, stationary phase cells were the most complex. This resembles the situation with C1300 neuroblastoma, where under normal conditions cells with long neurites are rare except in stationary phase cultures^{2,10}.

The C1300 neuroblastoma characteristically responds to the removal of serum and the presence of dibutyryl adenosine 3',5'-monophosphate (dibutyryl cyclic AMP) by rapidly extending neurites¹²⁻¹⁴. Although dibutyryl cyclic AMP induces morphological transformations in most cells, the effect of serum-free medium on the mouse neuroblastoma seems to be specific. If this response is restricted to cultured nerve cells, only nerve lines should respond to the removal

of serum with similar morphological transformations. Table 1 shows that this was the case. Thus the morphological response to low serum concentrations seems to distinguish neuronal from non-neuronal cells in this system. As expected from work on the mouse neuroblastoma and primary cultures of nervous tissue¹⁵, all the neuronal and most of the other cell lines were transformed morphologically in the presence of dibutyryl cyclic AMP (Table 1). A more detailed account of these changes will be presented elsewhere.

Neurotransmitter synthesis

Another distinction between the classes of cells in the nervous system is provided by the enzymes involved in neurotransmitter metabolism. The activities of choline acetyltransferase, tyrosine hydroxylase, glutamic acid decarboxylase, and both acetyl and butyrylcholine esterase were measured in stationary phase cultures as detailed elsewhere¹⁸⁻¹⁹. Table 1 shows that esterase activity was widely distributed among the cell lines, varying greatly with respect to both absolute amount and the ratio of activities on acetylthiocholine and butyrylthiocholine substrates. Al-

though B6, B19 and B104 have relatively high ratios, demonstrating true acetylcholine esterase, no cell lines approached the activities of C1300 neuroblastoma clones. Choline acetyltransferase is also widely distributed among the cell lines. It has been suggested, on the basis of indirect evidence, that glia synthesise acetylcholine²⁰; the data in Table 1 indicate that some of the non-neuronal cell lines in our collection have the requisite enzyme. Amino-oxyacetic acid-inhibitable glutamic acid decarboxylase activity was restricted to the neuronal cell lines, while γ -aminobutyric acid (GABA) was found in all of the putative nerve and glial lines. Although there was no strong correlation between glutamic acid decarboxylase activity and the ability of the cells to retain GABA, these data again suggest that glial cells can synthesise neurotransmitters. Tyrosine hydroxylase was found in B65 and at least two of the non-neuronal cell lines. With the exception of choline acetyltransferase activity in B65, the data indicate the enzyme specific activities for stationary phase cells only. These activities and the concentrations of the putative neurotransmitters vary as a function of the growth cycle (B. K., H. T., D. S., manuscript in preparation).

As Table 1 shows, several neuronal cell lines contain more than one neurotransmitter synthetic enzyme. For example, B65 contains choline acetyltransferase, tyrosine hydroxylase and glutamic acid decarboxylase activities; B35 and B103 both contain choline acetyltransferase and glutamic acid decarboxylase activities. Although it is generally assumed that an individual nerve cell makes but one transmitter, the evidence for this is not complete. In particular, it is not known if the newly formed nerve cell can synthesise a spectrum of neurotransmitters before it becomes functionally coupled with another cell. Possibly the shift toward predominantly one transmitter is a result of this type of cell-cell interaction, an event not documented in these clonal cultures. Finally, it should be pointed out that the specific activities of the enzymes involved in neurotransmitter metabolism in the cell lines are lower than those observed *in vivo* (Table 1). Since transmitter enzymes may be concentrated at functional nerve endings, and their levels regulated by trophic interactions between cells, the relatively low activities presented here may again reflect a lack of such interactions in these cultures.

Proteins unique to the nervous system

Of several proteins which seem to be localised in the nervous system, S100 and 14-3-2 are thought to be associated with glia and nerve, respectively^{21,22}, in spite of exceptions²³⁻²⁶. Since stationary phase cells exhibited the greatest morphological complexity seen during the growth cycle, they were used for the quantitation of these proteins. (It should be pointed out, however, that the intracellular concentrations of some enzymes and other proteins depend on the stage of the growth cycle both in these and in other cell lines^{27,28}.) To assay for S100 and 14-3-2, cells were homogenised and the soluble cell fraction was assayed by an indirect radio-immune procedure using iodinated antigen and antisera prepared against the pure antigen²⁹. Assays were routinely linearly sensitive between 1 ng and 100 ng for S100 and between 10 ng and 1000 ng for 14-3-2. (Concentrations of 14-3-2 and S100 in whole brain determined by this procedure were similar to those reported using other procedures^{30,31}.) Table 1 indicates that these proteins are not uniquely associated with either neuronal or non-neuronal cells. Although all the neuronal lines contain detectable 14-3-2, both proteins seem to be distributed independently among the remaining lines. Cells with undetectable levels of both proteins may not be of nerve or glial origin, an observation compatible with the fibroblast-like morphology of most cell lines in this group. These cells, which represent approxi-

mately 25% of the induced tumours, have not (with the exceptions of B1 and B56) been included in Table 1. However, cells which contain S100 or 14-3-2 and are not electrically excitable are of probable glial origin²¹⁻²⁶, and the presence of either protein cannot distinguish nerve from glia. Finally, all the cell lines of Table 1 were screened for neurophysin by the methods described for S100 and 14-3-2; none was found.

Some of the cell lines were also screened for secreted hydroxyproline, a marker for collagen³². Cells were labelled with ¹⁴C-proline and the secreted protein assayed for 4-hydroxyproline³³. Table 1 shows that all the cell lines examined secreted hydroxyproline. Although there was no absolute correlation with cell groups, these data suggest that more nerve-like group 2 cell lines secrete relatively less hydroxyproline-containing protein than the other morphological groups. This may explain the characteristic loose adhesion of the group 2 cells to the culture dish.

Conclusions from these data

We have drawn the following conclusions from our data.

- (1) Neoplasms of both neuronal and glial origin can be induced by established procedures in BDIX rats, and the resultant cells can be adapted to clonal cell culture.
- (2) At least 4% of our neoplasms yielded permanent nerve cell lines.
- (3) Nerve and glial cells can apparently be distinguished in cell culture on the basis of the effect of serum-free medium on cell morphology. All nerve and most glial cells undergo morphological changes in the presence of dibutyryl cyclic AMP, but only the nerve cells extend processes in the absence of serum.
- (4) Detectable glutamic acid decarboxylase activity is restricted to nerve lines, while low levels of GABA are also found in glial lines. Choline acetyltransferase and tyrosine hydroxylase activities are found in both nerve and glial cells. These results suggest that glia can synthesise neurotransmitters.
- (5) Some clonal nerve cell lines contain the enzymatic activities required to synthesise more than one neurotransmitter.
- (6) The nervous system proteins S100 and 14-3-2 are found in various combinations in both nerve and glial cell lines. All nerve cells contain detectable 14-3-2 while not all putative glial cells contain detectable S100.

Neuronal and non-neuronal lines can be distinguished on the basis of membrane excitability and neurotransmitter synthesis, but the sorting of non-neuronal lines has been difficult. On the basis of S100 and 14-3-2 protein synthesis, it can be argued that most of the cell lines presented in Table 1 are of either nerve or glial origin. It has been impossible, however, to group the putative glial cells on any basis except morphology. More specific criteria are needed.

Because all the cell lines in Table 1 derive from independently arising CNS tumours, they have greater functional diversity than is possible using one neoplasm. For example, clones of C1300 neuroblastoma have many different properties³⁴ but probably the original tumour was derived from a single cell, and the various stem cells resulted from later mutation and chromosome rearrangement¹⁰. In contrast, the rat nerve cell lines are not far removed from the original neoplastic cell, their karyotype approaches normal, and the clonal lines seem stable. Functional diversity and genetic stability are required if clonal lines are to be used with maximum effectiveness in the study of the mechanisms underlying the function of the mammalian nervous system.

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Lunar magnetism and an early cold Moon

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Models of lunar magnetism have involved dynamo action in a fluid core in an early hot Moon; an early cold Moon magnetised some time before 4.0×10^9 yr ago, which has subsequently heated up; and local field sources which, in some models, are related to impact. Here we examine the second possibility and show that provided the Moon contained a few percent of metallic iron and was exposed to an extra-Lunar field of about 10 or 20 oersted while much of it was still below the Curie point of iron, a restricted class of thermal evolution models, which satisfy the known constraints, can be derived.

ONE model of the thermal history of the Moon (see ref. 1-6) which has gained wide acceptance, involves a Moon which accreted cold but which was heated from the outside during the final stages of accretion. This would have given rise to a molten shell very early on in lunar history, and formed the crust which is indicated by seismic studies⁷⁻⁹. The interior of the Moon, however, would have been cool during its early history and would have warmed up because of radioactive heating only relatively recently, thus forming the molten or partially molten core which is indicated seismically⁷⁻⁹. Early

papers accounting for the magnetic field of the Moon suggested an initially hot Moon in which an iron core formed. Although moment of inertia constraints require that the core is small, such a core would at least behave as a magnetohydrodynamic fluid even at the present slow rotation rates¹⁰. There are, however, many difficulties¹¹ and here we consider whether an early hot outside, cold inside Moon, which evolved into the present cold outside, hot inside Moon, could quantitatively account for the evidence of an ancient magnetic field.

Origin of early magnetisation

If the Moon accreted cold then any metallic iron which was present may have acquired a remanent magnetisation from any of several mechanisms, provided there was an ambient field¹². We consider a mechanism in which the Moon was magnetised by an external steady field, thus acquiring isothermal remanent magnetisation (IRM). If the Moon were uniformly magnetised, it would need to have had a dipole moment of about 10^{23} gauss cm³ to give an ancient surface field of 2,000 gamma (γ) which is typical of several palaeointensity studies, although a much higher value has been reported¹³⁻¹⁶. This is well above the present value of less than 10^{20} gauss cm³ (ref. 17). We have measured the IRM of iron-bearing, igneous rocks from the Moon,

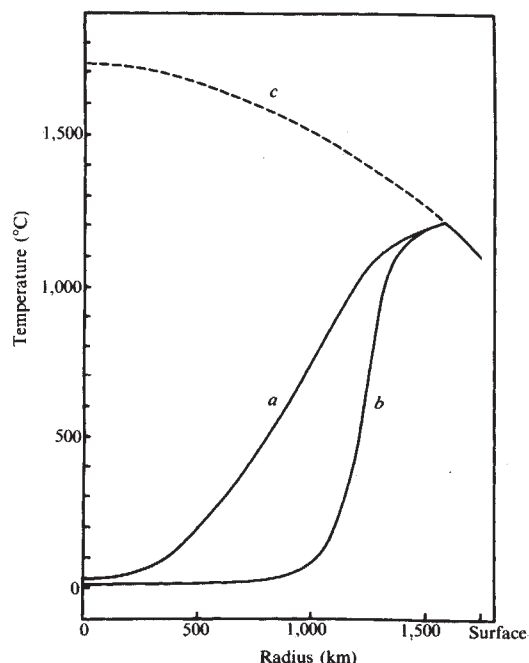


Fig. 1 Initial temperature distributions for an accreting Moon. The modified version permits the bulk of the Moon to remain below the Curie point of iron until 3.2×10^9 yr ago. *a*, Mizutani; *b*, modified; *c*, solidus.

which best represent the magnetic behaviour of rocks at depth. These samples generally have a high proportion of multidomain (coarse) iron and readily acquire a soft IRM in a weak field. On the other hand, the soils are rich in superparamagnetic ($\sim 300\text{\AA}$) iron, and the breccias range from superparamagnetic, unwelded samples, to multidomain, highly recrystallised samples. Typically, surface igneous rocks, which contain about 0.05 weight % metallic iron, acquire an IRM of about 3×10^{-5} e.m.u. g^{-1} in a 10 oersted field¹⁸. By extrapolation, similar material with about 3% by weight of iron would acquire an IRM of about 2×10^{-3} e.m.u. g^{-1} in a field of 10 oersted, giving the required dipole moment. Large variations in the distribution of iron in the Moon, both laterally and vertically, could give rise to significant local variations in the field; perhaps accounting in part for the diversity of palaeointensity determinations.

Table 1 Parameter values used for lunar thermal models^{1-6, 28}

Model	Mass ratios Th/U	K/U	Source	Heat generation ($\times 317$ erg $\text{g}^{-1} \text{ s}^{-1}$)	Decay constant ($\times 3.17 \times 10^{-18} \text{ s}^{-1}$)
Chondritic	4.15	80,000	U^{238}	2.97	1.54
Terrestrial	3.84	10,000	U^{235}	18.0	9.71
Lunar	4.00	2,000	Th^{232}	0.82	0.499
			K^{40}	0.94	5.5

Heat capacity, $cp = 10^7$ erg $\text{g}^{-1} \text{ } ^\circ\text{C}^{-1}$; heat of fusion, $hf = 4.0 \times 10^9$ erg g^{-1} ; density, $\rho = 3.34$ g cm^{-3} ; index of refraction, $n = 1.7$; opacity, $e = 100$ cm^{-1} ; Stefan-Boltzmann constant, $s = 5.669 \times 10^{-5}$ erg $\text{cm}^{-2} \text{ K}^{-4} \text{ s}^{-1}$

Two questions need to be answered. First, what is the origin of the magnetising field? Second, once the Moon had become magnetised by an isothermal process could the memory have been retained until the termination of igneous activity about 3.2×10^9 yr ago. There is no definitive answer to the first question, because there are no clear records of planetary fields predating those frozen into the lunar rocks. It is, however, possible to speculate either that such fields existed early

in the history of the Solar System in connection with local plasma instabilities, or that a close approach of the Moon to the Earth could have increased the turbulent motions in the Earth's core to the point at which the Earth's dynamo was much stronger than at present¹³. In either case a field of the order of 10 oersted needs to have existed, at least momentarily, some time after the bulk of the Moon had accreted.

The question of the time decay of IRM is hard to answer but extrapolation of the decay rate of IRM acquired in the laboratory in multidomain, iron-bearing rocks shows that the decay coefficient is very small²⁰.

Models of lunar evolution

A successful evolutionary model which will permit the Moon to retain an IRM long enough to provide a magnetic field in which the mare basalts would cool must also satisfy the following constraints.

(i) Early melting of the near surface regions, so that a crust may form by differentiation.

(ii) At least local melting in the near surface region between 3.2 and 4.0×10^9 yr ago, so as to provide a source for the mare basalts²¹.

(iii) Cessation of this near surface melting no later than 3.2×10^9 yr ago because, first, there seems to have been no igneous activity after this time, and, second, the outer portion of the Moon must have cooled sufficiently to support the mascons which formed probably no later than this date.

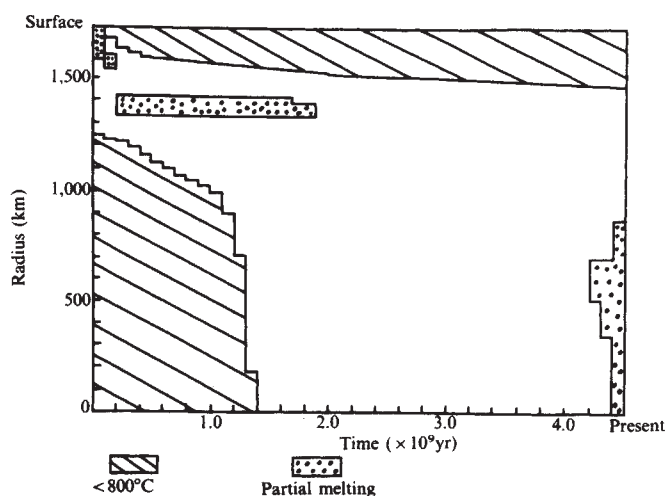


Fig. 2 Graph of radius against time showing the regions of partial melting and those regions which have temperatures below the Curie point of iron. For this model, the present-day surface heat flow is 23 erg $\text{cm}^{-2} \text{ s}^{-1}$.

(iv) Much of the volume of the Moon must have been below 770°C (the Curie point) until 3.2×10^9 yr ago so as not to remove the magnetising field.

(v) A present day partially (or totally) molten core with a radius not exceeding 850 km, as suggested by seismic data⁷⁻⁹ and predicted by Gast and Giuli²⁴.

(vi) The present day heat flow should lie in the range 20 – 30 erg $\text{cm}^{-2} \text{ s}^{-1}$ to be consistent with the Apollo 15 and 17 results²⁵.

In addition we have assumed that the Moon was of uniform composition at the time of accretion. We recognise this as an *ad hoc* assumption but chose to retain this simplicity until the model required otherwise. The basic initial temperature model (Fig. 1a) comes from Mizutani²³ and is close to his 'most likely' model, with $N_0 = 10^{-8}$ g cm^{-3} and $C = 3$ m s^{-1} , where N_0 is the

initial cloud concentration and C is the most probable particle speed for accretion. Using this particular model, the outer 150 km would be molten at the end of accretion and thus the Moon could have differentiated to give the present day crust.

The thermal history of the Moon was calculated using numerical methods but the analytical method was restricted to conduction solutions. The calculation is inherently nonlinear, because of the inclusion of a radiative term dependent upon temperature in the thermal conductivity. Details of the calculations will be published elsewhere.

The thermal conductivity used was $K + (16n^2s/3e)T^3$ where K is the lattice conductivity, T is the temperature and e the opacity (other terms are defined in Table 1). The parameters K and e are known from laboratory experiments to depend on temperature but in the range encountered in the models used here this is only a small effect²⁶. The value of K was allowed to range from 0.1×10^5 to 10×10^5 erg cm⁻¹ s⁻¹ °C⁻¹. The parameters used for n and e are given in Table 1.

Radioactive abundance ratios characteristic of lunar terrestrial and chondritic materials were used (Table 1). Our model does not account for any ratio changing upon fractionation. The values of uranium concentration were allowed to vary from 0.5×10^{-8} g g⁻¹ to 10×10^{-8} g g⁻¹.

The calculations accounted for partial melting, total melting, differentiation of heat sources, resolidification and remelting, and convection of heat in fluid zones. The melting point criterion was that of Ringwood and Essene²⁷.

The Mizutani version (Fig. 1a) produced a Moon which was well above the Curie temperature for most of its history. We therefore arbitrarily reduced the interior temperature until suitable models could be found (Fig. 1b). This model implies that the outer part of the Moon accreted somewhat more rapidly, and/or the inner part more slowly than is implied by Mizutani's model. Alternatively it means that the initial temperature was less than 0° C.

The models which satisfied the constraints were almost unique in the choice of conductivity and uranium concentration. This is because of the conflicting requirements that the interior of the Moon remain cold enough not to destroy its IRM during approximately the first 10^9 yr, but that it should heat up rapidly enough during the next 3×10^9 yr so as to produce a partially molten interior out to the present radius of ~850 km. Only a very delicate balance between the thermal conductivity and uranium concentration could satisfy both constraints under the general assumption of an initially chemically and physically uniform Moon. If we relax the constraint of an early cold Moon, most of which is below the Curie point, until about 3.2×10^9 yr ago, models which satisfy the remaining five constraints can be produced. The uranium concentration is about 50 parts in 10^9 , the thermal conductivity about 9×10^5 erg cm⁻¹ s⁻¹ °C⁻¹ and the heat flow value about 22 erg cm⁻² s⁻¹.

The thermal conductivities tend to be slightly higher than typical experimental values for representative lunar analogues²⁸. The uranium abundances are, however, very close to the average values commonly quoted for terrestrial, chondritic and lunar materials¹⁻⁶. Finally, the indicated heat flow values all fall within the range of values determined by the lunar heat flow experiments²⁹. Figure 2 illustrates that most of the Moon must remain below 770° C until about 3.2×10^9 yr ago.

We may now construct a sequence of events for the magnetic history of the Moon (Fig. 3). Initially the Moon was magnetised by a field of the order of 10 oersted when most of it was below the Curie point. The differentiation of the crust then occurred, concentrating a crust rich in anorthosite. Next, soils and breccia flows were formed primarily as a result of impacts and/or volcanic activity which enriched them in metallic iron. At or about the same time the mare basalts were formed. Finally, about 3.2×10^9 yr ago, the interior of the Moon heated up sufficiently to destroy the internal IRM, leaving only a magnetised crust. The crust could, in principle, still carry a dipole moment. The fact that none is observed

could be because the Moon may have been magnetised after the crust was formed. It may also be because the crust is formed of material such as anorthosites or anorthositic gabbros which carry only a weak magnetic remanence¹⁸. Subsequent impacting may also have randomised directions in the crust.

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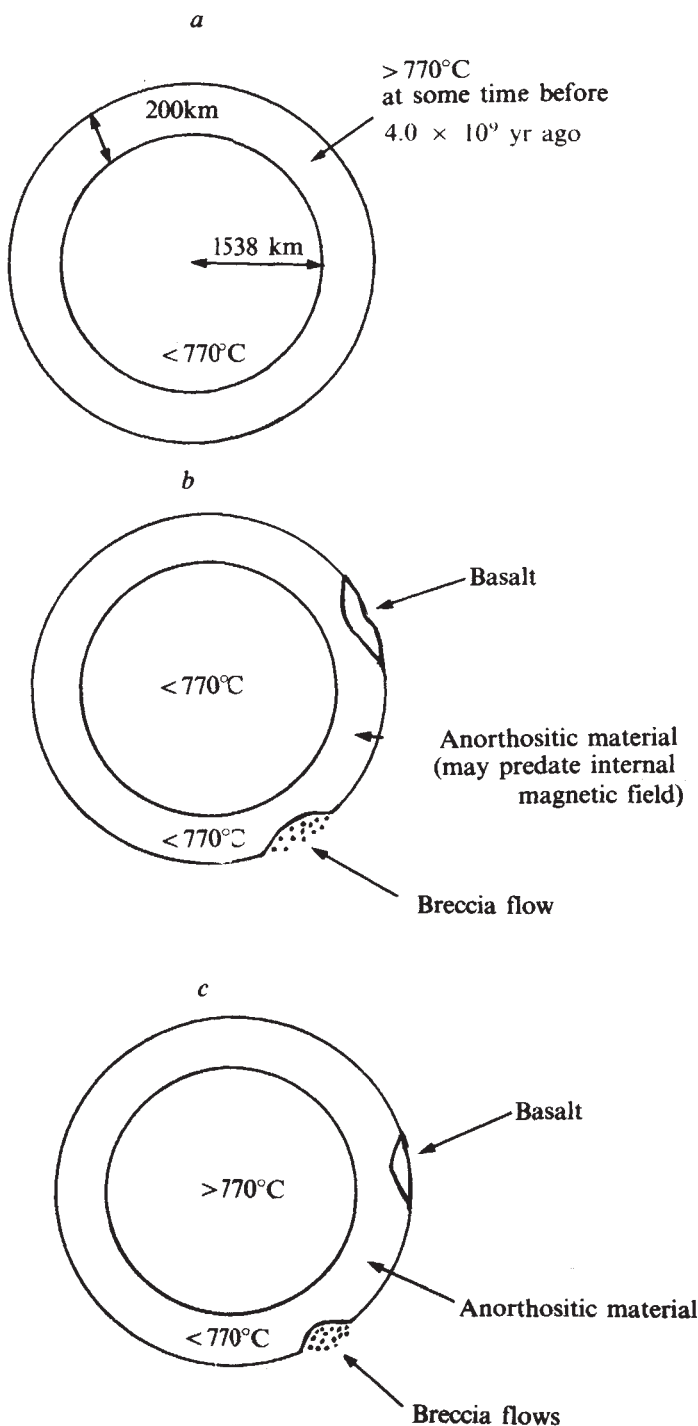


Fig 3. Schematic representation of the stages in the evolution of a cold Moon. The model presented here permits the magnetic, thermal, mean density, and moment of inertia constraints to be satisfied: a, before 4.0×10^9 yr ago; b, $4.0 - 3.2 \times 10^9$ yr ago; c, 3.2×10^9 yr ago.

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Gravitational deflection of polarised radiation

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An upper limit is determined for the difference in the deflection of beams of orthogonally polarised radiation passing through the Sun's gravitational field. A null result is anticipated by present theories of gravitation but this prediction has never been tested.

PRESENT theories of gravitation tacitly assume that two orthogonally polarised beams of radiation will be deflected by equal amounts on passing near the Sun's limb. This hypothesis has never been tested, even though available techniques should permit measurement of a differential deflection up to 10^4 times smaller than the total deflection suffered by the radiation.

The equivalence principle predicts that orthogonally polarised radiation should show no differences in deflection by the Sun. An observer falling freely into the Sun should see all beams of radiation following straight trajectories at his own location. If radiation polarised in one sense were deflected at a rate different from oppositely polarised radiation, then initially coincident beams of mixed radiation would separate into two beams that curve away from each other. At least radiation polarised in one of the two senses would no longer appear to propagate along a straight line for a freely falling observer.

Most theories of gravitation incorporate the equivalence principle, at least in the limit of geometrical optics and weak fields, and we would expect gravitationally deflected radiation to follow paths unaffected by the state of polarisation. But we can think of situations in which polarisation must play an important role. One particularly limiting case, pointed out to us by Dr Stuart Shapiro, involves maximally rotating black holes.

Such a black hole is incapable of absorbing long wavelength radiation approaching along the hole's axis of rotation if the

radiation is circularly polarised with spin angular momentum parallel to the angular momentum of the hole. This is because absorption of this radiation would endow the hole with excessive angular momentum and would add too little rest mass to the hole. As a result this radiation is not absorbed at all. In contrast, oppositely polarised radiation is readily absorbed since it merely tends to slow down the rotation of the black hole.

Although this picture is quite extreme, we would expect some measure of differentiation between left and right polarised radiation whenever long wavelength, circularly polarised radiation is scattered or absorbed by a rapidly rotating compact object (ref. 1 predicts a small Faraday-like rotation of the polarisation vector near a rapidly rotating mass). For shorter wavelengths, and larger, less rapidly rotating objects, these effects diminish, so that we again retrieve the result that gravitational effects should clearly distinguish different states of polarisation only in the diffraction limit, namely where the wavelength becomes comparable to the Schwarzschild or Kerr radius of the massive object.

We may eventually hope to make wavelength and polarisation-dependent observations that show such gravitational effects in eclipses of compact binary stars. But for the moment we have been attempting to establish, by observations, whether or not orthogonally polarised—linearly or circularly polarised—components of radiation are deflected by identical amounts in the Sun's gravitational field. Here we are clearly in the classical geometrical optics limit, and we are dealing with very low angular momentum in the deflecting mass. We would therefore expect no measurable difference in the deflection of orthogonally polarised radiation components.

Using very long baseline radio interferometry, this null result can, in principle, be determined with very high accuracy. The principal limitations are provided by the length of the available

baseline and Faraday rotation in the solar corona.

It is well known that the orthogonal polarisation components of a radio wave may be affected differently in propagation through a magnetised plasma. Faraday rotation of the plane of polarisation and plasma birefringence could obscure any differential gravitational deflection. For the present experiment the interplanetary plasma is expected to give the largest effect due to the Faraday rotation. We calculated, however, that even this Faraday rotation is negligible for solar elongations larger than $5R_{\odot}$. No significant plasma effects are expected from the ionosphere. Very long baseline interferometry, using a transcontinental baseline, offers a potential accuracy of almost 10^{-4} arc s at 8 GHz—of the order of one part in 10^4 of the total deflection.

If any effect is present, then the position of a small diameter radio source when viewed in one polarisation will be displaced from the position viewed with the orthogonal polarisation. This will show up as a slight change in the interferometer fringe rate between the two polarisation channels, an effect analogous to the beating of the separate components for a multicomponent source when viewed with a single polarisation.

As we are only searching for a relative displacement, the quantity to be measured is the relative phase difference between the orthogonal polarisations from the same radio source. This is independent of the local oscillator phase at each site provided that the same signal is applied to the two receivers at each radio telescope. In this way the full resolution of the interferometer can be realised.

Effectively, the observations search for an apparent splitting of the source, into orthogonally polarised components, as it approaches the Sun. Such a split can be detected far more readily than an absolute deflection relative to another radio source placed some degrees away in the sky. This second situation is the one that describes measurements of the normal deflection of radiation by the Sun. There the total change in angular separation of the sources 3C273 and 3C279 is measured to an accuracy of a few parts per million to arrive at a total deflection known to an accuracy of a few per cent.

Observations

The observations were carried out with the Green Bank-Owens Valley baseline on September 30 (day 274) and October 4 (day 278), 1972, when the radiation from these sources passes near the solar limb. The baseline is 3,900 km (10^8 wavelengths), and is nearly east-west. The Mark II VLBI tape recording system was used. The observations in Green Bank were with the Howard E. Tatel 85-foot (85-1) radiotelescope of the National Radio Astronomy Observatory (NRAO), and the frequency standard was obtained from the hydrogen maser installed at the 140-foot dish. At the Owens Valley Radio Observatory (OVRO) both A and B 90-foot radiotelescopes were used, and a Sulzer crystal oscillator, phase locked to a Hewlett Packard 5065A frequency standard, was used. Synchronisation was obtained from Loran C radio transmissions at NRAO and from the Goldstone Tracking Station at OVRO.

Different polarisations were sampled by switching 85-1 between left and right circular polarisation (LCP and RCP) every 2 s, while switching between antennae A and B at OVRO every 1 s. Antennae A and B had linearly polarised feeds, which were preset to be orthogonal. Hence in a period of 4 s all four combinations of polarisation were sampled. As the coherence time of the rubidium-driven Sulzer oscillator is longer than the switching rate, relative phases between the different polarisations were easily obtained. The preliminary reduction was accomplished with the NRAO Mark II processor, and the fringes corresponding to the various polarisations were sorted by computer and combined into their respective groups. The VLBI interferometer was sensitive to dual sidebands of 2 MHz width centred at 8,075 and 8,095 MHz. Each sideband was processed separately.

In Fig. 1 the relative positions of 3C273, 3C279, and the Sun during the experiment are shown. Unfortunately, the signal-to-noise ratio for 3C279 was too small to allow us to make any convincing measurements of relative phase, although weak fringes were detected. This is due to the weak correlated flux² in 3C279 (≤ 3 f.u.). The correlated flux in 3C273 was about 12 f.u., and the relative phases between orthogonal circular polarisations were easily measured. On the two days of observations 3C273 was 5.0° and 7.8° from the Sun.

We have not been able to determine the phase difference in orthogonal linear polarisations due to a discrepancy, as yet not understood, in fringe rate between antennae A and B (although the change in baseline was properly taken into account). But two independent determinations of phase differences in the circular polarisations were obtained from separate analyses of the fringes produced by correlating antenna 85-1 with antenna A, and by correlating 85-1 with antenna B. For a source separated into two components, with angular separation, $\Delta\theta$, at position angle A , the fringe phase difference is $\Delta\Phi = \Delta\theta [(B_x \sin A - B_y \sin \delta \cos A) \sin H - (B_x \sin \delta \cos A + B_y \sin A) \cos H + B_z \cos \delta \cos A] + \text{constant}$

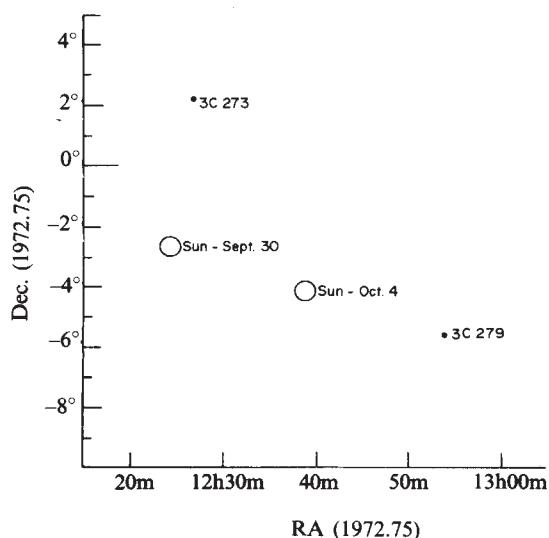


Fig. 1 Positions of 3C273, 3C279 and the Sun on the observing days.

where H is the hour angle, (α, δ) is the source position, and B_x , B_y , and B_z are the baseline components in wavelengths. Symmetry considerations lead us to expect that any separation of circular polarisation components will be perpendicular to the radial direction. Thus the position angle of the splitting, A , is orthogonal to the position angle from the Sun to the radio source. Phase differences between the orthogonal circular polarisations were calculated for scans of 15 min during the observing sessions. The data shown in Fig. 2 were fitted to the equation by the method of least squares. This was done for the B antenna experiment on October 4 and for the A antenna experiment on both days. The B antenna was not functioning properly on the first observing day. The phase differences for the upper and lower sidebands have been kept separate, since the exact times of relative phase determination, and the values of B_x , B_y and B_z differ slightly. Also the data from both days in the A antenna experiment were, in one case, taken together in the same least squares fit by arbitrarily assuming the splitting, $\Delta\theta$, to have a $1/r$ dependence on solar elongation. None of these least squares fits yielded significant splitting above the error level. Upper limits for all of these cases were obtained by calculating χ^2 for assumed values of $\Delta\theta$. The upper limits of the splitting, corresponding to the 95% confidence level are given in Table 1. It is noted that by assuming a yet stronger radial dependence

Table 1 Upper limits to the polarisation splitting

Polarisation	Observing days used	Data used	Splitting upper limits (95% confidence)				
			Tangential		Radial		
			Solar elongation ($R_{\odot}$)	$\Delta\theta$ clockwise (10^{-3} arc s)	$\Delta\theta$ counter- clockwise (10^{-3} arc s)	$\Delta\theta_{in}$ (10^{-3} arc s)	$\Delta\theta_{out}$ (10^{-3} arc s)
Circular	278	Even	7.8	2.4	1.9	4.1	3.3
Circular	278	Odd	7.8	2.2	0.5	—	3.0
Circular	274	Even	5.0	27	25	336	467
Circular	274 and 278	Even	7.8	2.1	1.5	3.7	3.5
Dependence on R neglected							
Circular	274 and 278	Even	7.8	1.6	1.1	3.5	3.4
1/R dependence assumed							
Linear	Change of polarisation technique		4 to 6			2.5×10^{-4}	2.5×10^{-4}
Circular	Faraday rotation technique		7			2.1×10^{-3}	2.1×10^{-3}

Clockwise tangential splitting is defined as that seen by an observer in which right circular polarisation appears rotated clockwise about the Sun away from left circular polarisation. In outward radial splitting right circular polarisation appears at greater solar elongation than left circular polarisation. The 'even' data are the result of correlating antennae A and 85-1, and the 'odd' data from correlating antennae B and 85-1.

than $1/r$, smaller upper limits are obtained since additional weight is effectively put on the data obtained at smaller solar elongations.

Results for circular polarisation

Symmetry considerations suggest that two circularly polarised radiation components could at best be split along a line perpendicular to the direction of the (non-rotating) deflecting mass.

As shown in Table 1, our observations set an upper limit of roughly 0.002 arc s for the difference in the deflection of the two circularly polarised components along a direction perpendicular to the solar radius vector. Along the direction of the radius vector, our upper limit is approximately 0.0035 arc s.

These upper limits are to be compared to a total gravitational deflection of ~ 0.2 arc s for radiation passing at these distances from the Sun.

Linearly polarised radiation

Radiation reaching us from a source that lies beyond the Sun can be decomposed into two orthogonally linearly polarised radiation components, with one polarised in a direction parallel to the radius vector from the Sun and the other polarised along a tangential direction. If these two components are not deflected identically by the Sun's gravitational field, it can be because one of the two components is slowed down more on approaching the Sun. This produces splitting along the radial direction. This situation is consistent with symmetry considerations. The component with the slower velocity is deflected more strongly, since its wavefront is retarded more strongly in the Sun's vicinity.

One way of measuring the time delay due to such a differential reduction in the velocity of propagation of the wave is to search for phase delays in the propagation of the two components. If the radially polarised component R and the tangentially polarised component T are equally strong, and the source is initially linearly polarised in a direction at 45° to the solar radius vector, then any difference in the propagation time past the Sun would make itself felt as a transition toward elliptical polarisation. If the difference in propagation time became as large as one quarter of a cycle, the initially linearly polarised wave would become purely circularly polarised. At a delay of half a cycle the wave would again be linearly polarised, but at an angle 90° removed from the initial direction (Fig. 3).

Thereafter there would be a second stage of elliptical polarisation, culminating in circular polarisation for a delay

of $3/4$ cycle. But the sense of circular polarisation between delays of 0 and $1/2$ cycle is opposite to that between $1/2$ and 1 cycle. A delay by a full cycle gives a wave linearly polarised in the initial sense, as emitted at the source. The intermediate state of elliptical or circularly polarised radiation would be right handed or left handed depending on which of the two components—the radial or the tangential—was propagated less rapidly.

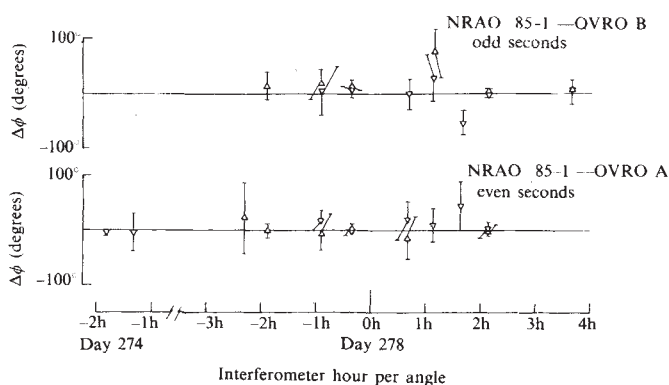


Fig. 2 Relative fringe phase between left and right circular polarisation for 3C273 is shown as a function of hour angle. Each point corresponds to approximately 15 min of observing. Upper (Δ) and lower (∇) sidebands are plotted separately. The arbitrary additive phase constant has been removed.

Observations carried out by Stelzried *et al.* in 1968 on the plane polarised carrier wave of 2.29 GHz (13 cm) transmitted by the solar orbiting spacecraft Pioneer 6 followed the source to within about $4R_{\odot}$ from the Sun. Although the polarisation emitted by the spacecraft was almost purely tangential to the solar radius vector³, strong Faraday rotation was observed when the transmitted beam passed within about $6R_{\odot}$ from the Sun. By $4R_{\odot}$, where the signal became too difficult to follow, the polarisation angle had rotated from 90° —perpendicular to the ecliptic plane—through 0° and on to -45° .

It is virtually certain that at some time during this period Faraday rotation would have produced a polarisation angle of $\sim 45^\circ$ near the ray's closest point of approach to the Sun. Nevertheless, no ellipticity was detected, although the instrumental resolution of power axial ratio was ~ 0.1 . This implies a maximum phase shift between the two linearly polarised components amounting to much less than 1 cm in separation. In contrast, a wave passing within $4-6R_{\odot}$ of the Sun is⁴ delayed $\sim 10^6$ cm, indicating that the time delay and its associated bending should be identical for both linear polarisations to within one part in $\sim 10^6$.

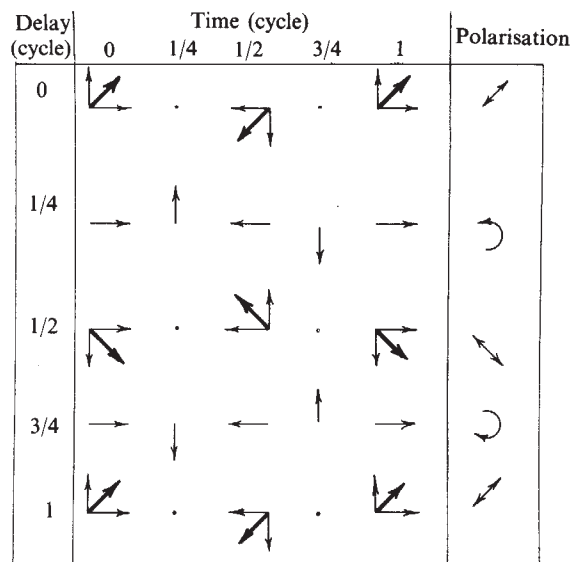


Fig. 3 The polarisation of a wave formed by the superposition of two orthogonal linearly polarised waves. The resulting polarisation depends on the phase relationship or delay between the two waves.

Beyond $7R_{\odot}$, where Faraday rotation effects seem to be negligible, the Pioneer 6 experiment also gives an upper limit on the time delay difference for orthogonally polarised circular components. This appears to be less than about one part in 10^5 of the total effect.

Space probe experiments of this type in the X band or at higher frequency could provide more stringent results.

The future

We have been able to place upper bounds on the possible difference in the deflection of orthogonally polarised radiation components passing through the Sun's gravitational field. Although these bounds are already quite stringent, order of magnitude improvements can be expected within the next few years by straightforward improvements in the techniques described here. Without extensive innovations, we may expect sensitivities to reach one part in 10^4 to 10^6 of the total gravitational deflection. Even a null result obtained in such observations should be a useful constraint for the construction

of theories of gravitation.

We mentioned earlier that there should exist differential deflection effects that depend not only on polarisation but also on wavelength. These effects may be too small to measure in the deflection of radiation passing by the Sun, but we may hope to obtain significant results in astronomical observations involving eclipses of compact binary stars.

Very long baseline interferometer observations such as ours may also eventually have application to the polarisation structure of compact radio sources. Indeed one model of expanding doubles predicts that each component will have weak circular polarisation orthogonal to the other (unpublished work of R. H. Sanders). For our purposes polarisation structure can be separated from any gravitational effects by observing each source at different solar elongations. The observed lack of circular polarisation⁵ in 3C273 implies that any structural features that could be detected should be much smaller than 0.001 arc s.

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Note added in proof:

After this paper had been submitted, our attention was drawn to a paper by Mashhoan⁶, in which the questions discussed here are placed into a general relativistic framework. The author derives several important results that help in predicting the size of expected effects.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Dust in the head of Comet Kohoutek

WE have been making photographic observations of Comet Kohoutek (1973f) at the observatory of Izaña, Tenerife, with the aim of assaying the dust content of the tail. We have taken many photographs on various films and with various filters; having enlarged and printed some of the negatives we have noticed on many of them a straight dark streak running away from the head for a distance of about $2\frac{1}{2}$ arc deg. The streak is to be seen on exposures made on 1974 January 9, 10,

11 and 12 on Kodak Tri-X Pan, RAR 2495 and Eastman Kodak High Speed Infra-red (HIE-135) emulsions and with focal lengths of 85, 135 and 225 mm (Fig. 1).

We conjectured that the streak might be the shadow of the head of the comet cast on to the tail and so we computed the position angle of the Sun with respect to the comet head. We found that it agreed with the streak direction to within our limits of accuracy of measurement (about 10 arc min). We assumed that our objectives were free from distortion and that the photographs represented a stereographic projection of the celestial sphere centred on the comet's head.

If this explanation of the streak is accepted, several interesting conclusions follow:

(1) The opaque region casting the shadow has a diameter

of about 4×10^7 m (25,000 miles). This follows from the observed length of the streak and the known distance of the comet from the Earth at the time. It does not follow, of course, that there is a solid nucleus of this diameter since even sparse dust in sufficient depth can cause enough opacity.

(2) The opacity is caused by dust, not gas, and this dust has a low albedo, otherwise the comet would have been much brighter.

(3) If we assume as usual, that the particle size distribution obeys a power law $n(a) = ka^{-p}$ with $p = 3$ for small particles¹ and 4 for larger ones, and that the space density of particles varies inversely as the square of the distance from the centre of mass (or nucleus if there is a nucleus) as required by a simple continuity equation, then it is possible to calculate what we might call the 'opacity radius'—the distance from the centre of mass at which a light beam must pass in order to be attenuated by a factor $1/e$. Alternatively if this radius is known then the mass enclosed within a sphere of this radius can be computed. A simple formula can be derived: $M = (2/3)apD^2$, where D is the opacity radius, a is the upper limit of particle radius and ρ is the material density of the particles. Here we have apparently $D = 2 \times 10^7$ m and $a = 10^{-4}$ m (ref. 2) since the power law seems to break down for large particles; and if $p = 3$ then $M = 8 \times 10^{13}$ kg; $p = 8$ gives $M = 2 \times 10^{14}$ kg.

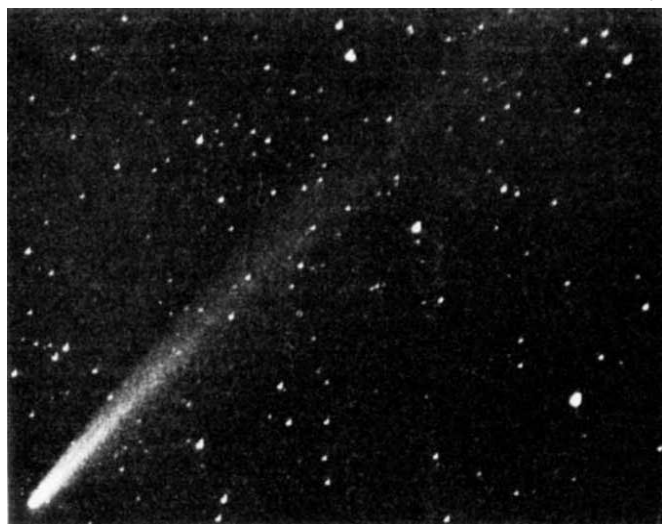


Fig. 1 Comet 1773f at 1974 January 9. In white light, exposure 80 s at $f/2$ on Kodak Tri-X Pan film. The head had a magnitude of $+3.5$ at this time. The three bright stars at the top of the field are (from left to right): 46 Capricorni, ξ Aquarii, β Aquarii.

These figures agree well with the estimates made by Whipple³ for the total mass of a comet and suggest the possibility that the whole mass may be contained in the dust cloud. There would be ample surface area, 3×10^{15} m² if the particles are spherical, more if they are not, for the adsorption of gases and sufficient opacity to prevent them from volatilising too quickly.

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Atmosphere of Mercury

THE recently released news concerning the possible atmosphere surrounding Mercury seems to have taken the astronomical community by surprise. It had generally been expected that because of the high temperatures (of the order of 450 K) existing there and also because of the low surface gravity, any atmosphere originally present, or any released from subsequent events on the surface would quickly evaporate since the random thermal speed of the atoms would be greater than the escape velocity. If V_E is the escape velocity from Mercury, ρ_A the density of its atmosphere and A the surface area, then a simple computation indicates^{1,2} that the rate of mass loss from this atmosphere is

$$\frac{dM}{dt} = \frac{A\rho_A}{\sqrt{\beta\pi}} [1 + \beta V_E^2] \exp(-\beta V_E^2)$$

where $\beta = m/2kT$, k being Boltzmann's constant and m the atomic mass. All this ignores the solar wind which carries mass into the atmosphere. In reality the problem becomes a boundary layer problem in which the magnetic field of Mercury plays a part, but we can see simply that the main effect is to generate an atmosphere. If the wind has a density ρ_w and a velocity V_w , then it adds matter to Mercury at the rate

$$\frac{dM}{dt} = AV_w \rho_w$$

and the steady state situation is given when

$$V_w \rho_w = \frac{\rho_A}{\sqrt{\pi\beta}} [1 + \beta V_E^2] \exp(-\beta V_E^2)$$

$$\text{or} \quad \frac{\rho_A}{\rho_w} = \left[\frac{\sqrt{\pi\beta} V_w \exp(\beta V_E^2)}{[1 + \beta V_E^2]} \right]$$

Inserting the appropriate numerical values, $\beta \approx 10^{-11}$, $V_E^2 \approx 2 \times 10^{10}$, and $V_w \approx 10^8$ (all in c.g.s. units gives)

$$\frac{\rho_A}{\rho_w} = 600$$

There should therefore be a very tenuous atmosphere, basically composed of hydrogen being maintained in this way. Any helium and argon released by radioactivity would become trapped in this atmosphere and should be detected.

If the atmosphere is significantly denser than given above, it implies that volcanic activity or some such gas generating process must be active now.

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Existence of two groups in thermoluminescence of meteorites

THE thermoluminescence (TL) properties of meteorites¹⁻⁴ may be applicable to the determination of exposure age, terrestrial age, thermal gradients, radiation history and preatmospheric shape. In an attempt to apply TL to the determination of terrestrial age we have discovered that meteorites can be divided into two groups, the existence of which is relevant to several of the applications.

Our apparatus consists of a molybdenum strip heated electrically at $5 \pm 0.05^\circ \text{C s}^{-1}$ by an electronic control unit. The light emitted by 10 mg of 50 μm sieved powder placed on a defined area of the strip is measured by a cooled and shielded 9635B EMI photomultiplier tube. The amplified signal is plotted automatically against temperature. The resulting 'glow curve' has two peaks, one at about 200°C (LT) and one at about 350°C (HT).

Meteorite	Terrestrial age (yr)	BM no. (if applicable)	Class	Thermoluminescence group
Barwell	8		L6	Low
Bruderheim	13		L6	Low
Khanpur	21		LL5	Low
Mangwendi	39		LL6	Low
Olivenza	49		LL5	Low
Saratov	55	BM1956, 169	L4	High
Crumlin	71	BM86115	L5	Low
Gambat	76	BM83864	L6	Low
Jelica	83		LL6	Low
Soko Banja	96		LL4	High
Tennasilim	101	BM1913, 218	L4	High
Butsura	112	BM34795	H6	High
Dhurmsala	113		LL6	Low
Aldsworth	138	BM61308	LL5	Low
Durala	158	BM32097	L6	High
Limerick	160		H5	Low
Wold Cottage	178	BM1073	L6	High
Mauerkirchen	205	BM19967	L6	High
Ogi	232	BM55256	H6	High

We recorded the glow curves of nineteen meteorites that were seen to fall and are therefore of accurately known terrestrial age (Table 1). Two groups were immediately apparent; one in which LT falls below HT in about 100 yr after fall, and one in which it takes several hundred years for this to occur. Although the two groups are evident by visual inspection of the glow curves they are more obvious in Fig. 1, a plot of $\log_e$ (peak height ratio, LT/HT) against the terrestrial age of the meteorite. The low retentivity group, in which LT is fading fastest, shows much scatter from the line but the high group shows surprisingly little. The cause of the scatter is probably small initial differences in the TL of the meteorites, different thermal histories since the decay is temperature sensitive and, in the case of the low group, varying degrees of shock.

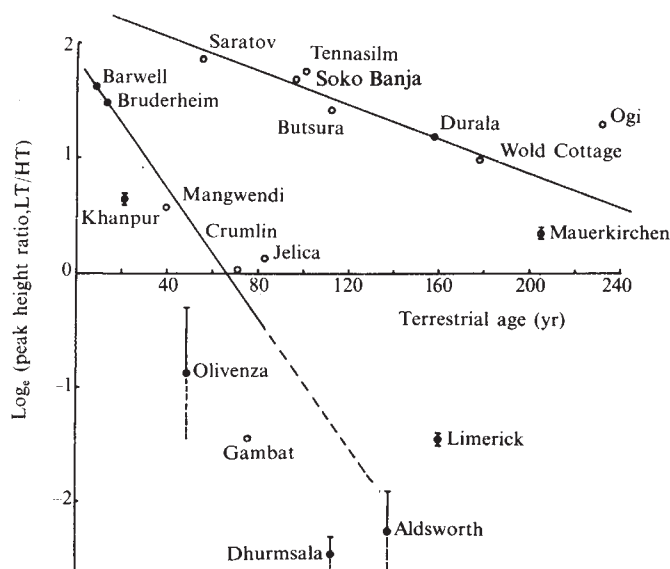


Fig. 1 Natural logarithm of the peak height ratio (LT/HT) as a function of terrestrial age, for meteorites of known terrestrial age.

To confirm that meteorites in the low retentivity group are fading faster than meteorites in the high retentivity group, the mean life (time to fall to $1/e$) of LT in six meteorites was determined. We heated powder from three high and three low group meteorites in the dark at 120°C for various periods up to 70 h. Heating was done in an Abderhalden drying pistol surrounded by refluxing tetrachloroethylene. The results are shown in Fig. 2. Although the curves are not exponential, and

the results must be treated as approximate, it is clear that the two groups are due to different mean lives of LT in the luminescent material. The mean life, τ , at room temperature can be calculated from

$$\tau(300\text{K}) = \tau(393\text{K}) \exp [(E/k300) - (E/k393)]$$

where E , the energy of the peak, can be found by the initial rise method and k is the Boltzman constant⁵. For the low retentivity group the mean life at room temperature is 6–8 yr and for the high group 45–55 yr.

We have not been able to determine with certainty the reason for the two groups but suspect that it is related to shock. Both metamorphism and shock change the nature of the luminescent feldspar, shock producing maskelynite and metamorphism causing recrystallisation. Chemical-petrological groups assigned on the extent of metamorphism⁶ show no correlation

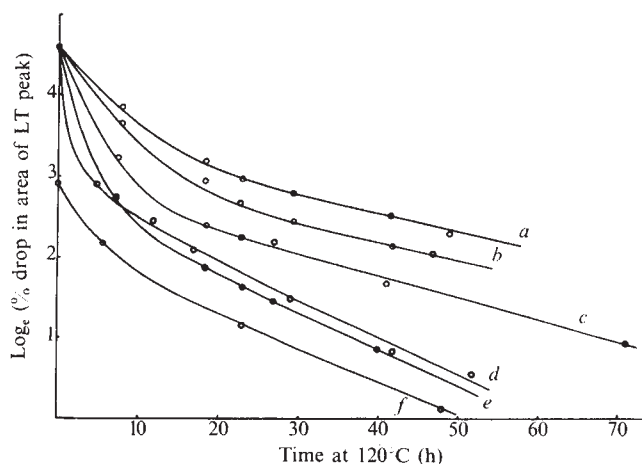


Fig. 2 Isothermal annealing curves for three high and three low retentivity meteorites. The ordinate is the natural logarithm of the percentage drop in the area under LT. Overlap has been allowed for where necessary and the curve for Jelica has been displaced vertically downwards for clarity. a, Wold Cottage (50–54 h); b, Saratov (50–55 h); c, Tennasilim (50–55 h); d, Barwell (28–34 h); e, Bruderheim (26–34 h); f, Jelica (28–34 h). The numerical values are estimated for the mean lives at 120°C .

with the TL groups of the meteorites, inferring that metamorphism does not govern TL group membership. Shock is known to reduce TL intensity, however, and we have reason to believe that it also reduces the mean life of LT. Most of the members of the low group have been shocked or are breccias (R. Hutchison, personal communication). Olivenza and Barwell are anomalous in this respect, as is Soko Banja which is in the high group but is a breccia. Shock may also explain the scatter in the low group line in Fig. 1 since it can occur with various degrees of severity.

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Fission track dating of a vitric tuff from East Rudolf, North Kenya

ACCURATE dating of the Plio-Pleistocene sedimentary sequence in the East Rudolf basin of northern Kenya is urgently required to provide a time framework for the hominoid^{1,2}, archaeological^{3,4} and other^{5,6} important finds in the area. A sequence of water-lain tuff horizons are intercalated with lacustrine, deltaic and fluvial sediments⁷ and if a number of these tuffs can be dated with sufficient accuracy they could provide both the primary geochronological framework required and, by establishing correlations between East Rudolf sections and the world-wide geomagnetic reversal time scale, enable palaeomagnetic dating to be applied to those parts of the stratigraphy lying between the dated tuff horizons.

⁴⁰Argon/³⁹Argon age spectrum analysis by Fitch and Miller⁸ has provided a firm date of 2.61 ± 0.26 Myr for the prominent KBS Tuff horizon that occurs near the middle of the sedimentary sequence. This date is compatible with the probable age ranges of the sediments suggested by palaeontological correlation with dated sequences elsewhere in East Africa⁹ and with the initial findings of the palaeomagnetic reversal study⁹. Further work by Fitch and Miller⁸ indicates that straightforward K/Ar or ⁴⁰Argon/³⁹Argon dating of the East Rudolf tuff horizons is complicated by episodes of enhanced groundwater metasomatism which probably resulted from the periodic release of volcanic heat and/or hot fluids in the area. They suggest that although the East Rudolf succession contains clear evidence of a sequence of local, explosive ignimbritic eruptions over the period 5.0 to 0.5 Myr, in some parts of the sedimentary basin metasomatism has disturbed the argon and potassium isotope ratio of the component crystal and glass fractions of the tuffs produced by these eruptions in such a way as to cause partial and complete overprinting of the apparent ages obtained from them. The repeated occurrence of the same discrepantly low ages from different tuffs at different locations is explained as resulting from a series of distinct, short-lived overprinting events, rather than the postulation that the discrepancies result from the accumulative effect of prolonged, low energy geochemical activity in a diagenetic environment. One of the most widespread of the overprinting events recognised by Fitch and Miller occurred around 1.75 Myr (ref. 10).

We first investigated the feasibility and practical problems involved in applying fission track dating to East Rudolf tuffs and then tried to confirm or deny the presence of a widespread thermal annealing event around 1.75 Myr. The sample used was a vitric tuff from the upper part of the Kubi Algi Formation, inland from Allia Bay in the south-east of the East Rudolf basin (Kenya Grid Reference BH72001640). The tuff was collected by A. K. Behrensmeier and V. J. Maglio in 1970 from section B5. A minimum age of 3.0 Myr was suggested by Maglio for the Kubi Algi formation; Fitch and Miller have proposed an age of around 3.9 Myr based on conventional K/Ar and ⁴⁰Argon/

³⁹Argon age determinations. More precise dating was precluded by the very complex age spectrum obtained; analysis of the spectrum indicates both partial contamination by an older volcanic fraction included in the tuff and also minor discrepancies in argon loss caused by subsequent events.

Petrologically the sample used for this track study is an unconsolidated volcanic ash, largely composed of glass shards but also containing abundant calcite grains, small amounts of biotite and feldspar, and numerous minute grains of iron oxide. Only the glass shards can be regarded as definitely juvenile; microscopic examination shows the shards to be fresh, strongly vesicular and bearing no sign of devitrification.

The fission track dating method¹¹ and, more specifically, the use of volcanic glass in track dating¹²⁻¹⁵ have been described previously. Uncontaminated glass shards were separated from the ash sample by hand picking beneath a binocular microscope; the shards were divided into two aliquots. One aliquot was heated to 250° C for 52 h, which was observed to anneal the spontaneous tracks present; it was then irradiated using a thermal neutron fluence of approximately 1×10^{14} neutrons cm⁻². The fluence was carefully monitored using NBS standard glass SRM614, with a uranium content of 0.82 p.p.m., allowance being made for the depleted ²³⁵U. The natural and irradiated shards were simultaneously mounted in epoxy resin, polished and then etched in 2.75 M hydrofluoric acid for 25 min at 20° C. The two aliquots were consecutively counted using an eyepiece graticule in a Reichert Zetaplan microscope; incident illumination with a magnification of $\times 256$ was used. The natural shards revealed tracks formed by the spontaneous fission of ²³⁸U and the irradiated shards showed tracks resulting from the neutron-induced fission of ²³⁵U.

Table 1 shows the resulting spontaneous and induced track densities; it presents an apparent fission track age of 1.8 Myr. No precise value for the error has been attached, since errors quoted in previous studies have been of questionable value. Considering the track counting, fluence dosimetry and the uncertainty of the value of the ²³⁸U fission decay constant, a minimum value of 10% is given for the uncertainty of the date. The variation in track density between shards was about 20–30%, indicating a relatively homogeneous uranium distribution. Since fission tracks in glass are particularly susceptible to thermal annealing¹⁶, the mean track diameters of both the induced and spontaneous tracks were measured. Comparison of these results revealed no significant difference in track diameter, indicating that the specimen has either suffered no thermal annealing or that it has been totally annealed at 1.8 Myr.

As this tuff is within the Kubi Algi Formation and is stratigraphically below the 2.6 Myr KBS Tuff, the second alternative is accepted as the correct interpretation. Thus this study has shown that fission track dating of Plio-Pleistocene vitric tuffs from the Lake Rudolf area is practicable and that in appropriate circumstances this method can produce evidence of important events in the

Table 1 Spontaneous and induced track densities of vitric tuffs.

Sample and location	No. spontaneous tracks counted (1)	Spontaneous track density cm ⁻² p _s	No. induced tracks counted (2)	Induced track density cm ⁻² p _i	Fission track date (3)
AJH64 Kubi Algi SB5-R3	403	2.558×10^5	987	5.871×10^5	1.8×10^6 yr

(1) Total tracks in 100 natural shards; an area of 1,681 μm² counted in each shard.

(2) Total tracks in 100 annealed irradiated shards; an area of 1,681 μm² counted in each shard.

(3) Neutron fluence = 8.59×10^{13} neutrons cm⁻²; induced track density in dosimeter glass 518 tracks cm⁻², 89 tracks counted in 10,000 fields; spontaneous fission decay constant for ²³⁸U taken as 8.42×10^{-17} yr⁻¹ (ref. 17).

history of the rocks, other than or in addition to the age of initial volcanism. The apparent age obtained from the sample studied indicates that it was subjected to thermal annealing around 1.8 Myr and this can be taken as additional confirmation of the geologic reality of the important overprinting event around 1.75 Myr, indicated by previous K/Ar and $^{40}\text{Ar}/^{39}\text{Ar}$ studies¹⁰.

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Oxidation effect on the analysis of iron in the interstitial water of recent anoxic sediments

RECENT studies on the changes in composition of interstitial water during its separation from sediments¹⁻³ prompted us to evaluate the effect of squeezing on the concentration of ferrous iron within the interstitial water. Sediment samples were taken in northern Chesapeake Bay (38°56' N and 76°25' W) with a Benthos gravity corer. Layers of sediment were immediately extruded from the plastic coring tube and loaded into nylon squeezer barrels. The interstitial water was squeezed from the sediment through a 0.22 μm Millipore filter by the pressure of nitrogen acting against a rubber diaphragm⁴. The water drained directly into a calibrated test tube containing a solution of *o*-phenanthroline, buffered at pH 3.6, and the iron content was determined colorimetrically⁵.

During attempts to determine the precision of the analysis, we discovered that the dissolved Fe(II) concentration in the interstitial water increased during the squeezing process. Figure 1a shows the Fe(II) concentrations in consecutive aliquots of 3 ml as a function of volume of interstitial water emerging from the squeezer. The shape of the squeezing curve, a rapid initial rise in concentration to a constant value, was observed in every subsequent experiment.

We investigated five factors which could cause the increase of the interstitial Fe(II) concentration: filter brand, filter pore size,

temperature and pressure of squeezing, and the presence of oxygen. In all of the experiments, excluding the one comparing oxygen-rich with oxygen-free conditions, all manipulations were carried out in a nitrogen atmosphere. During squeezing we found no significant differences in the concentration of dissolved Fe(II) in interstitial waters squeezed through Millipore, Gelman and Nucleopore filters, nor in waters squeezed through Millipore filters with pore sizes of 0.22, 0.10, 0.05 and 0.025 μm . Interstitial Fe(II) concentrations did not vary with squeezing temperatures of between 7 and 20° C nor with squeezing pressures of between 50 and 200 pound inch⁻². The only factor which appreciably affected the iron concentration was the presence of oxygen.

Although there have been several studies on the concentrations of trace constituents in the interstitial water of anoxic sediments⁶⁻⁹, nobody to our knowledge has attempted to prevent the oxidation of these waters by any means other than rapid handling of the sediment. We have evaluated the importance of oxidation. Nine cores were taken in rapid succession; a layer of each core, 67–72 cm below the sediment–water interface, was extruded, homogenised and loaded into two 2.5 cm squeezers containing deoxygenated filters in a glove bag from which oxygen had been purged with nitrogen.^{11,12} One of the pair of squeezers from each core was never exposed to

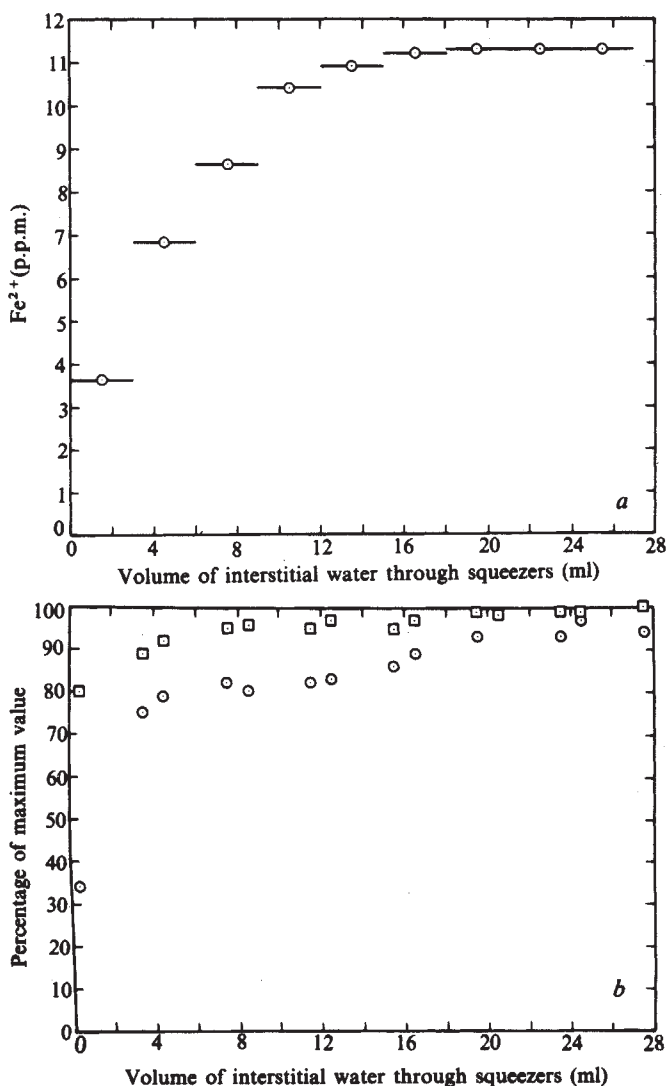


Fig. 1 a, Change in concentration of dissolved ferrous iron as interstitial water is squeezed. Horizontal lines indicate consecutive 3 ml aliquots analysed. b, Comparison of the changes in concentration during squeezing under nitrogen ($\square$) and air ($\circ$).

the atmosphere and was manipulated under nitrogen. The second squeezer of each pair was removed from the nitrogen glove bag after loading and exposed to the atmosphere for 15 min with its top removed. Consequently, the surface of the sediment and the top of the drainage tube were exposed to oxygen for a period of time approximating rapid manipulation of a sediment core. These latter samples were then squeezed in air. Squeezing began at the same time for both the exposed and unexposed samples. An aliquot was taken simultaneously from each of the squeezers after 15 ml of interstitial water had been squeezed. In eight of the nine cores the Fe(II) concentrations in the interstitial waters squeezed under nitrogen were greater than those squeezed in air; and in five of these eight, the difference between the exposed and unexposed samples exceeded 50%. The mean of the nine samples squeezed in air was 10.0 p.p.m.; in nitrogen, 12.9 p.p.m.

We repeated this experiment using a sediment core stored at 4°C. Successive 1 ml aliquots of the interstitial water passing through duplicate squeezers were analysed for ferrous iron in order to determine the change in concentrations as a function of volume. Figure 1b shows the concentrations plotted as a percentage of the maximum value. The results indicate that the difference in ferrous iron concentration of the interstitial water squeezed in air and in nitrogen occurs primarily during the squeezing of the first 15–20 ml. We conclude that discrete sampling and analysis of the aliquot directly from the squeezer, under a nitrogen atmosphere, is the best method of ensuring analytical accuracy.

Inorganic oxidation of dissolved ferrous iron is rapid in the neutral pH range, 6.8–8.0, typical of interstitial waters from Chesapeake Bay¹⁰. We observed a noticeable change in colour, from black to grey, extending several millimetres into the top and bottom of sediment samples extruded and squeezed in air. This same change in colour occurred in sediment samples exposed to air and is a result of oxidation¹¹. Sediment protected by a nitrogen atmosphere during the entire sampling operation remained black for the duration of the procedure.

The rapid change in Fe(II) concentration observed in the initial period of squeezing must result from the oxidation of ferrous ion to ferric hydroxide by the small amount of oxygen trapped in the squeezer during transfer of sediment from the core to the squeezer. As oxygen is consumed, the Fe(II) concentration levels off at its true value. In our samples oxidation significantly affected the concentration of Fe(II) up to values of 10–20 p.p.m. The effect on other trace metals whose concentrations fall in the parts per 10⁹ range, either directly through reaction with oxygen or indirectly through interaction with amorphous ferric hydroxide¹² would be even more pronounced. Acidification of squeezed samples does not alleviate the problem because the dissolved Fe(II) is oxidised and lost from solution within the squeezer. Thus, trace metal analysis of anoxic interstitial waters rich in iron must be performed in an inert atmosphere.

We used methods of oxidation protection in an investigation of the composition of interstitial waters from a station in northern Chesapeake Bay (Fig. 2a–f). Phosphate was determined colorimetrically and carbonate alkalinity titrimetrically¹⁴. The distribution of sulphide, Eh and pH were determined with electrodes. The core was sampled at intervals of 2 cm to a depth of 12 cm and then at intervals of 7.5 cm to a depth of approximately 100 cm.

Below a depth of 1 to 2 cm sediments from Chesapeake Bay are anoxic and the decomposition of organic matter enriches carbonate alkalinity and phosphate with depth in the interstitial water. The generation of sulphide from the concomitant bacterial reduction of sulphate is commonly assumed to immobilise iron as an iron sulphide. We observed, however, two layers of significant dissolved Fe(II) mobilisation into the interstitial waters throughout the sediments of the northern Chesapeake Bay: a narrow band between the surface and 10 cm, and a broad layer below 40 cm. The maximum concentrations of iron observed in the interstitial water, consistently between

500 and 1,000 $\mu\text{g atom l}^{-1}$ (28–56 p.p.m.), are between one and three orders of magnitude greater than the concentrations observed in anoxic sediments by other investigators^{6–9}. These

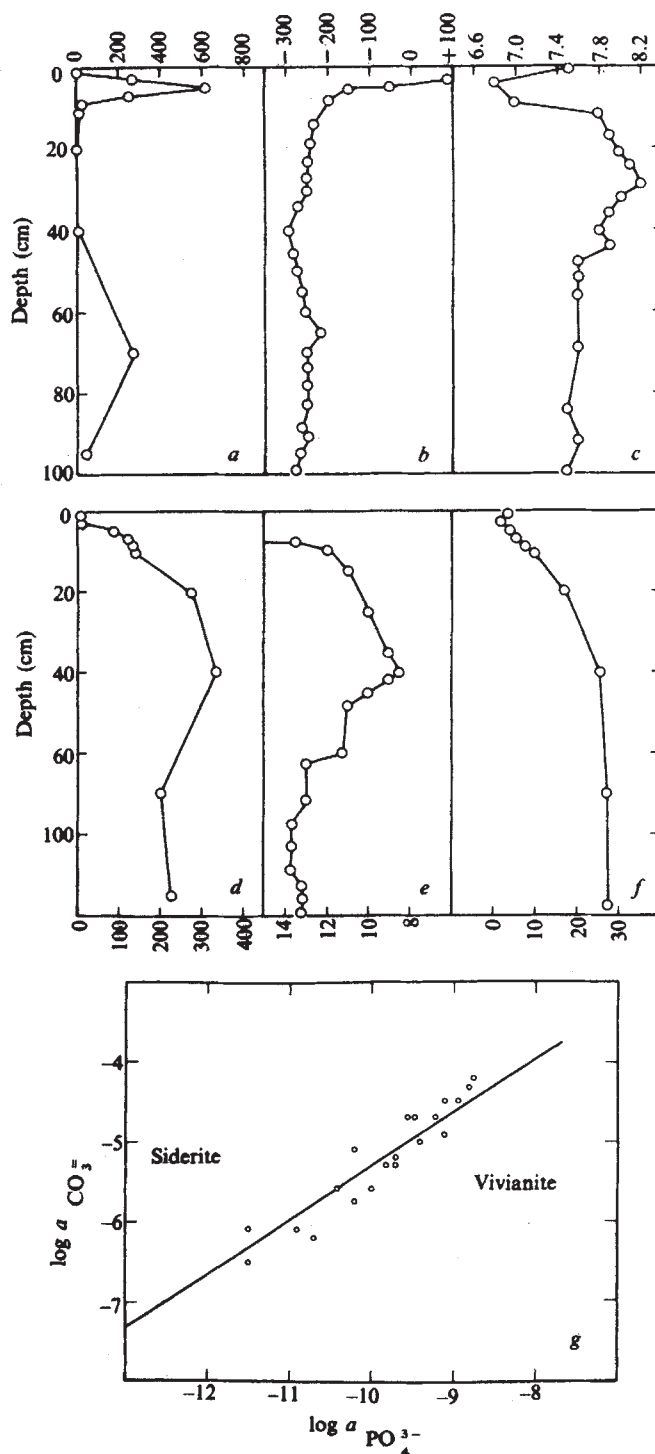


Fig. 2 Interstitial water chemistry at station 2, 38°56'08"N and 76°25'18" W; water depth 12 m. Figures a to f are plotted as a function of depth below the sediment–water interface. a, Fe²⁺ ($\mu\text{g atom l}^{-1}$); b, Eh (mV); c, pH; d, reactive phosphate ($\mu\text{g atom l}^{-1}$); e, PS_2^- ; f, carbonate alkalinity (meq l^{-1}); g, equilibrium among siderite, vivianite and interstitial water. The activities on the plot were calculated from the concentrations using an ion-pairing model and the Debye–Huckel equation. The speciation of all known inorganic ion pairs of importance was computed as a function of ionic strength from published stability constants; in this treatment, therefore, the activity of an ion is the product of the concentration of the free, uncomplexed ion and its individual activity coefficient.

unusually large concentrations may be due to the unique characteristics of the sediments of Chesapeake Bay or to the lack of sufficient oxidation protection in previous investigations. Between 2 and 5 cm below the sediment-water interface the strong positive iron gradient probably results from the equally strong negative *Eh* gradient. Between depths of 5 and 10 cm we can pinpoint no single unambiguous cause for the rapid decrease in the ferrous iron concentration, since the concentrations of sulphide, phosphate, and carbonate, all increased rapidly.

The analytically determined concentration of a dissolved species consists of the free ion concentration plus the concentrations of that species present in ion pairs and soluble complexes¹⁵. The product of the free ion concentration and the appropriate activity coefficient yields the activity of the species. Our computed activities of carbonate and phosphate are plotted in Fig. 2g, in which the line represents the theoretical equilibrium between siderite, FeCO_3 , and vivianite, $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ (refs 16, 17). The close correspondence between the data points and the equilibrium line suggests that in these pore waters the activities of Fe(II) , carbonate, and phosphate are co-dependent. Further, the close negative correlation between the Fe(II) and sulphide distributions suggests that sulphide may be controlled by iron in these iron-rich sediments.

In anoxic sediments, therefore, the distribution of Fe(II) in the interstitial water can be a sensitive indicator not only of general redox conditions but also of the chemical equilibria of certain nutrients. We conclude that the handling of anoxic sediments and the analysis of iron-rich pore waters must be carried out in an inert atmosphere if accurate results are to be obtained.

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Spirorbid — algal stromatolites

MODERN stromatolites may be formed in certain environmental settings by several different processes and organisms¹, although they are predominantly produced by blue-green algae. Algal stromatolites occur in the Lower Triassic Bunter formation (Buntsandstein) in the Krosno Odrzanski region of western Poland. Spirorbid tubes often occur in the immediate vicinity of these stromatolites (Fig. 1), which are usually found unattached. Because spirorbid tubes are generally attached to

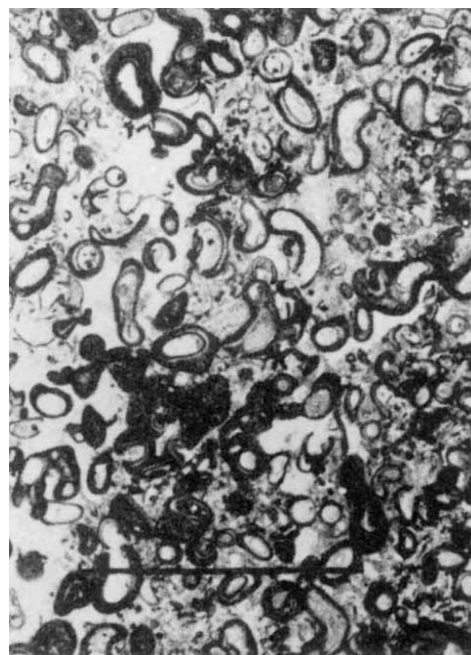


Fig. 1 Photomicrograph of organodetrritic carbonate. Fossils are predominantly spirorbirds. Some spirorbid tubes form oolitic nuclei. Scale bar, 1 cm.

submerged objects, mainly shells and pelagic and sedentary algae², such unattached occurrences seem to indicate that the spirorbirds lived on algae.

Associations of stromatolites and spirorbirds³ or serpulids⁴ have been found before. Garwood⁵ mentions that in the Lower Carboniferous Main Algal Series of Tuedian Beds in Cumberland and Roxburghshire, the surfaces of small domelike

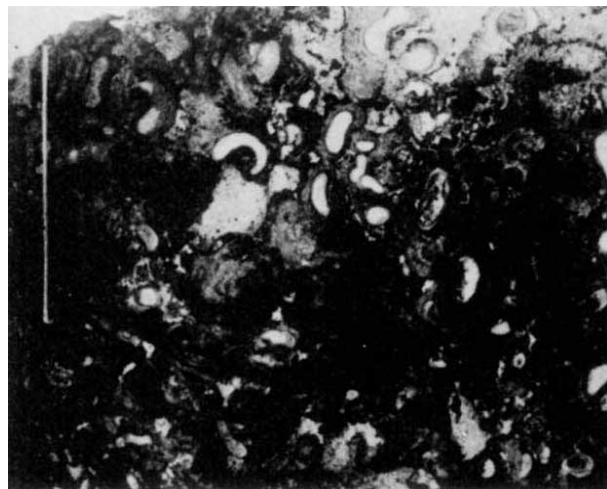
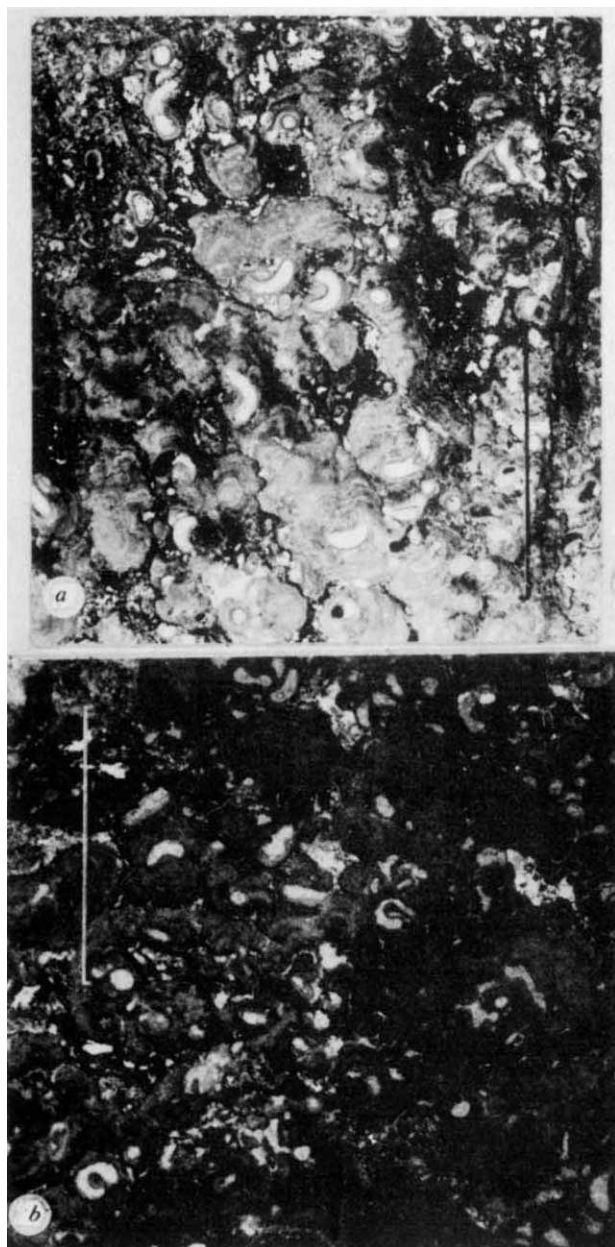


Fig. 2 Photomicrograph of spirorbid and algal-built stromatolites. Larger stromatolite fragments are found in any position. Subsequent algal laminae on the top of the debris. Scale bar, 1 cm.



Figs. 3 a and b Photomicrograph of spirorbid and algal-built stromatolites. Scale bar, 1 cm.

'protuberances' (stromatolites?) are frequently encrusted with adherent tubes of *Spirorbis*. Spirorbids also occur within the Bunter stromatolites (Figs 2 and 3). The stromatolitic structures developed on spirorbid tubes, and the detritus may contain small amounts of spirorbid fragments. As a rule, however, spirorbids occur in great number inside stromatolites (Figs 2 and 3) which are built not only by algae but also by spirorbids themselves. In contemporaneous worm-algal stromatolites from the Persian Gulf⁶ burrowing worms build mounds which may be coated with algae but the worms do not enter into the framework of the stromatolitic structure.

The unique nature of spirorbid-algal association in the Krosno Odrzanskie region is best explained as resulting from the particular environmental conditions prevalent during the deposition of the Bunter stromatolite-bearing rocks. The Bunter algal stromatolites originated in upper subtidal and lower peritidal zones. Stromatolites only occur sporadically in the upper peritidal zone. Tidal influence on sedimentation is reflected in changes of stromatolitic structures. These can best be interpreted as resulting from repeated periodic events:

influxes of coarse sediment, commonly evident in stromatolitic sequences, could have resulted from periodic catastrophic erosion and deposition. Crust-building communities lived in such tidal marine environments. Flakes and chips of stromatolites are common in the interareas of stromatolitic structures (Fig. 2). Larger stromatolite fragments also occur as clumps which have been rolled and are found in any position, commonly on their sides or upside down (Fig. 2). On top of the debris later colonies of algae may develop (Fig. 2). Stromatolitic intraclasts and characteristic sequences of stromatolitic structures indicate a high energy environment.

The spirorbid-algal community therefore seems to be an ecological adaptation to a rather high energy environment. The spirorbids probably reinforced the structure of spirorbid-algal stromatolites, which were therefore more resistant to the erosional effects of waves and currents than were ordinary algal stromatolites.

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Infrared Zimm plots for macromolecular characterisation

THE light scattering method is an accepted procedure for the measurement of the molecular weight (M) and the size of macromolecules in solution. This information is most readily available for particles the major dimension (ξ) of which is of the order of the wavelength (λ) of the light incident on the system. Provided that they are not too optically dense, such particles satisfy the conditions

$$|m-1| \ll 1 \text{ and } \frac{2\pi\xi}{\lambda} |m-1| \ll 1$$

and are described in terms of the Rayleigh-Gans-Debye^{1,2} (RGD) scattering theory. In this equation, m is the ratio of the refractive index (n) of the solute to that of the solvent (n_0).

If the particles are relatively small in comparison to λ (that is $\xi < \lambda/20$) then the simple Rayleigh^{1,2} theory is applicable but no information is obtained about the particle size. Alternatively, if the particles are too large the scattered intensity becomes an oscillating function of θ and complicated interference effects occur. For this case, a general solution has been developed³, but only for spheres.

With the improvement of sources and detectors which operate in the infrared, it is possible to increase the wavelength of scattering experiments so as to bring some particles which exhibit interference peaks in the visible region into the realm of the RGD theory, at least over a restricted angular range. Measurements can then be made on readily modified commercial apparatus and the data can be analysed according to the straight forward Zimm plot⁴ method. The radius of gyration (S) and M can then be directly determined, even with polydisperse samples. An alternative procedure⁴, that of measuring at extremely low angles θ to the forward direction of the incident beam, requires a purpose built apparatus.

We are at present engaged in a study of the scattering from aqueous suspensions of bacteria, with dimensions typically of the order 1–2 μm . Preliminary studies on *Serratia marcescens* provide an example. Figure 1 shows that this system exhibits

angular scattering peaks in the visible spectrum, even at the lowest values of θ accessible to a commercial scattering instrument. The source of our scattering photometer, a SOFICA 4200, was replaced by a continuous working Nd³⁺ YAG laser which operates at $\lambda = 1.06 \mu\text{m}$. The detector was replaced by a combination of an infrared converter tube (type EMI 9606) and a 13-stage photomultiplier (type EMI 9635QB). Infrared-sensitive photomultipliers are a simpler alternative to this arrangement. The light trap was modified so that measurements could be made up to $\theta = 10^\circ$ and an oscilloscope was used to record the photomultiplier response. These are all relatively trivial changes. The Zimm plot (Fig. 2) was then obtained from data for four different dilutions of the bacterial suspension. The experimental precision was commensurate with that of visible scattering experiments.

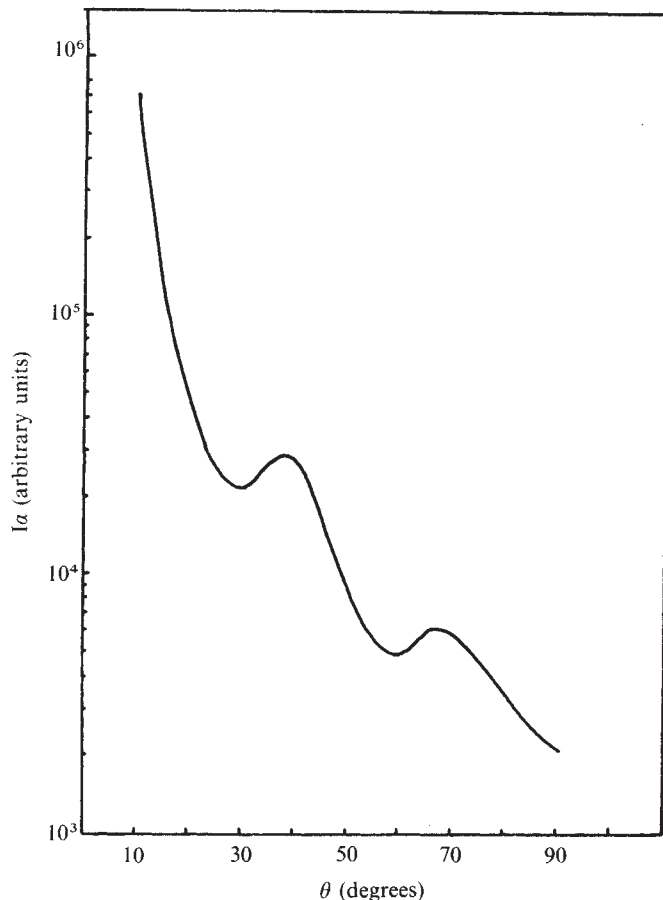


Fig. 1 Part of the angular scattering profile for *S. marcescens* in water. Data for $\lambda = 436 \text{ nm}$ and a suspension concentration of $2.4 \times 10^{-6} \text{ g ml}^{-1}$. Oscillations are present even at low angles. The factor $\alpha = \sin\theta (1 + \cos^2\theta)^{-1}$.

A value of 0.18 was assumed⁵ for the refractive index increment (dn/dc), necessary for the interpretation of the plot intercept¹. This value is strictly for the visible region of the spectrum and does not vary much with wavelength provided that no absorption band exists between visible wavelengths and $1.06 \mu\text{m}$. This was verified experimentally. We are developing a method for the direct measurement of (dn/dc) in the infrared region. From Fig. 2, values of $M_w = 1.0 \times 10^{11}$ and $(\bar{S}^2)^{1/2} = 0.34 \mu\text{m}$ were determined. The latter is equivalent to a spherical diameter $\bar{d}_s$ of $0.88 \mu\text{m}$. The subscripts refer to the relevant polydispersity averages. These values correspond to an average bacterial mass of $1.7 \times 10^{-13} \text{ g}$. This mass and the particle size agree with other experimental data for this system⁶.

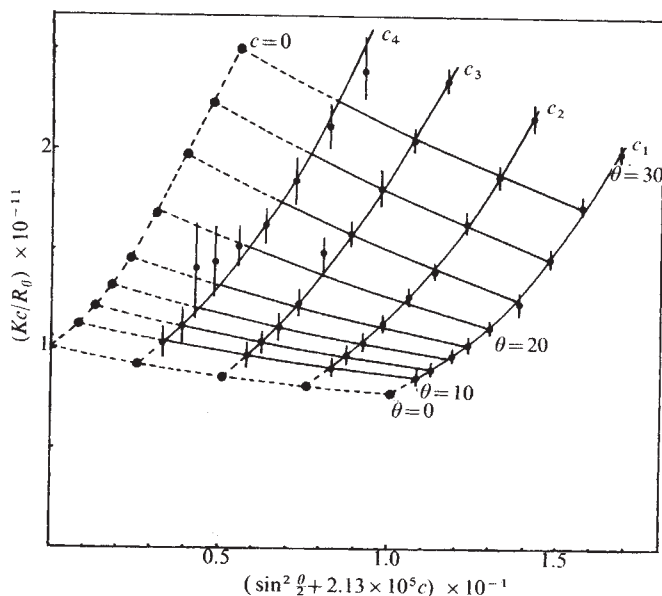


Fig. 2 Zimm plot for *S. marcescens* at $\lambda = 1.06 \mu\text{m}$. C was the stock concentration of $4.7 \times 10^{-7} \text{ g ml}^{-1}$. The dilutions were as follows; $c_1 = C$, $c_2 = 0.75C$, $c_3 = 0.50C$, $c_4 = 0.25C$.

We therefore conclude that with the advance in infrared lasers and instrumentation it is possible to adapt conventional, commercial scattering photometers to work in the infrared. Provided that neither solute nor solvent are absorbing at the selected wavelength, particles which are too large to be studied through the RGD scattering methods in the visible region of the spectrum conform to the regular Zimm plot procedure when the incident wavelength is in the infrared. Their molecular weights and sizes may then be evaluated with relative ease.

Finally, the method can be extended well beyond the wavelength of the present preliminary experiment. For example, by modifying the optics, the present experiments could be extended to the $10.6 \mu\text{m}$ wavelength of the CO₂ laser. Airborne particles could then be studied. The combination of both increased wavelength and low angle measurement is doubly beneficial.

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Fossils in mélanges

So-called 'tectonic' mélanges in many parts of the world seem to contain a disproportionately high number of fossils relative to the enclosing rocks. This is particularly true of mélanges in 'greywacke type' terrains, in particular the Esk Head Mélange¹ in the otherwise sparsely fossiliferous Torlesse Zone of the Alpine Assemblage² in the New Zealand Geosyncline, the mélange belts of the Franciscan Assemblage of California (M. C. Blake, jun., personal communication), and the Liptrap Formation of southern Victoria, Australia. In addition, the fossils contained in mélange belts commonly contain shallow water forms, and in extreme cases even terrestrial fossils (D. L. Jones, personal communication).

These facts alone seem to preclude postdepositional tectonic modification of part of a sedimentary sequence as the sole means of mélange formation. Rather, we suggest that the processes leading to the formation of at least some mélange belts resulted in the development of localised, specialised sedimentary environments during deposition, probably in shallow water and commonly accompanied by submarine volcanism. Continuation of the same tectonic regime, possibly in an accentuated or modified form, then led to the formation of mélange belts in parts of the deposited, but only partly consolidated, sedimentary sequence.

We fully support the suggestion³ that the term mélange should be used only in a descriptive sense and that the prefixes 'sedimentary' and 'tectonic' should be used with the utmost caution.

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Light scattering from soap films

If a strong light beam falls on the surface of a liquid, most of it will be reflected and refracted in certain directions, but a small part will be scattered in all other directions. This light scattering is caused by small surface ripples (corrugations) arising from thermal motion. The scattering intensity is proportional to kT/γ , where k is the Boltzmann constant, T the absolute temperature and γ the surface tension. In the case of a thin, free, liquid film (as found in soap bubbles), the scattered light also contains information about the interaction forces between the two film surfaces. According to the theory of Vrij¹, the second derivative, d^2F/dh^2 , of the interaction free energy, F , between the two film surfaces, with respect to the film thickness, h , can be obtained in this way.

When electrical double layer and van der Waals forces are operative in the film one may write¹ $d^2F/dh^2 = B\kappa^3 \exp(-\kappa h) - A/(2\pi h^4)$, where B is a function of the surface potential, κ is the reciprocal Debye-Hückel thickness and A is the van der Waals-Hamaker constant.

Although a few results have been published^{1,2}, no measurements that would corroborate the theory in a completely satisfactory manner have ever been reported. Here we present some results for films drawn from a solution containing 8.2×10^{-4} M cetyltrimethylammoniumbromide (CTAB) and 8.4 (weight) % glycerol. Experiments were carried out on draining films (varying h) at constant angles. From plots of $\ln[d^2F/dh^2 + A/(2\pi h^4)]$ against h , the values of κ were calculated. In the (very small) van der Waals term we used $A = 6.28 \times 10^{-20}$ J. From the values of κ , the electrolyte concentrations were calculated (Table 1) and compared with the concentration of the solution. The agreement between the two is good, in contrast with older experiments¹ where the calculated concentrations were up to 16 times too large.

Table 1 Results calculated from the thickness dependence of the light scattering (h between about 70 and 90 nm).

Angle of incidence	Angle of observation	κ (m ⁻¹)	Concentration of CTAB (mol l ⁻¹)
(°)	(°)		
60	55.0	9.78×10^8	8.6×10^{-4}
60	54.0	8.70×10^8	6.8×10^{-4}
60	53.1	1.03×10^9	9.6×10^{-4}

After drainage of a film to constant thickness, we also studied the angular dependence of the light scattering. In Table 2 the observed scattering is compared with values predicted by calculation using the results of the dependence on thickness of the light scattering. It is clear that the experimental results fit the predictions very well.

We conclude that the measurements of the thickness- and

Table 2 Angular dependence of the light scattering

Angle of observation	Predicted light scattering ratio	Measured light scattering ratio
(°)	($\times 10^6$)	($\times 10^6$)
54	6.56	6.69
53	5.86	5.37
52	4.41	4.42
51	3.68	3.78
50	3.10	3.30
49	2.64	2.66
48	2.26	2.34
46	1.69	1.78
44	1.30	1.37
41	0.91	0.97
37	0.60	0.63

angle-dependent light scattering show that the light scattering theory is internally consistent. Further, they show the validity of the exponential thickness dependence of the electrical part of d^2F/dh^2 .

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Role of molecular aggregates in liquid drag reduction by polymers

SMALL quantities of certain polymers dissolved in a liquid can reduce considerably the frictional drag exerted by the liquid when it flows over a surface. This effect has been observed with various solvent/polymer combinations, but most investigations involve polyethyleneoxide (PEO), polyacrylamide or guar gum in water, where the concentrations required are of the order of a few parts per million.

Although the mechanism of this drag reduction is unknown, attention has generally focused on the role played by the individual polymer molecules. There might also be entanglements or aggregates of polymer molecules¹, especially in freshly prepared solutions², and the breakup of these under shear, like the degradation of polymer molecules themselves³, may explain the frequently observed phenomenon of time-dependent drag reduction. But there has been little attempt to investigate these clusters, and most workers have been at pains to eliminate their effects. Recently, however, Hand and Williams⁴ have shown that in drag-reducing solutions there is an 'adsorbed-entangled' layer of polymer molecules at the liquid/solid interface and have suggested that this is responsible for much, if not all, of the drag-reducing behaviour. We report here studies⁵ of the time dependence of drag reduction in solutions of PEO and polyacrylamide which provide new evidence for the existence of such aggregates and indicate that they can be much more powerful drag reducers than the individual polymer molecules.

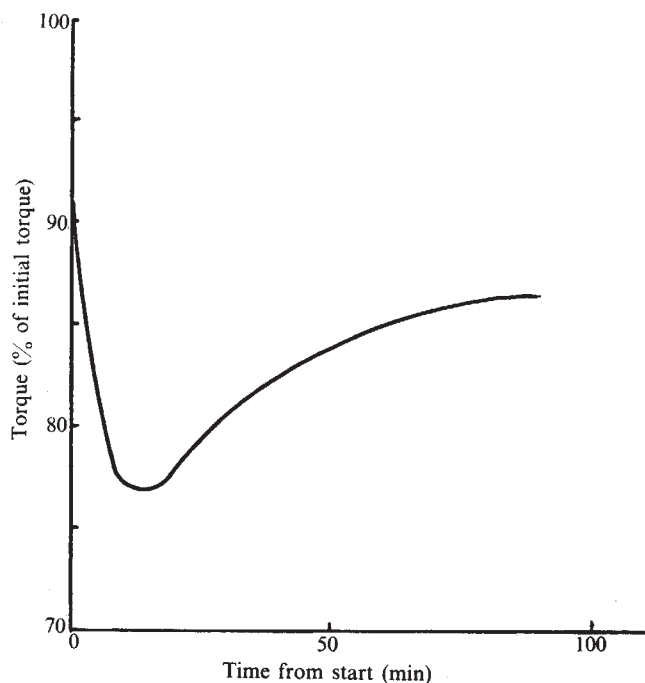


Fig. 1 Typical torque-time curve obtained with 'as received' (unsieved) material. Polyox Coagulant, 0.86 p.p.m., 840 r.p.m., 17° C.

The apparatus consisted of a vertical tapered stainless steel disk of diameter 0.23 m revolving, usually at 840 r.p.m. in a tank of water of volume 0.58 m³. A dynamometer attached to the shaft of the disk enabled torque, and therefore drag reduction, to be measured as a function of time. The desired quantity of PEO powder was added below the water surface as a suspension in isopropanol. Most experiments were carried out using Polyox Coagulant with a molecular weight greater than 5×10^6 .

The result of a typical experiment is shown in Fig. 1. The addition of the solid results in a rapid reduction of the required

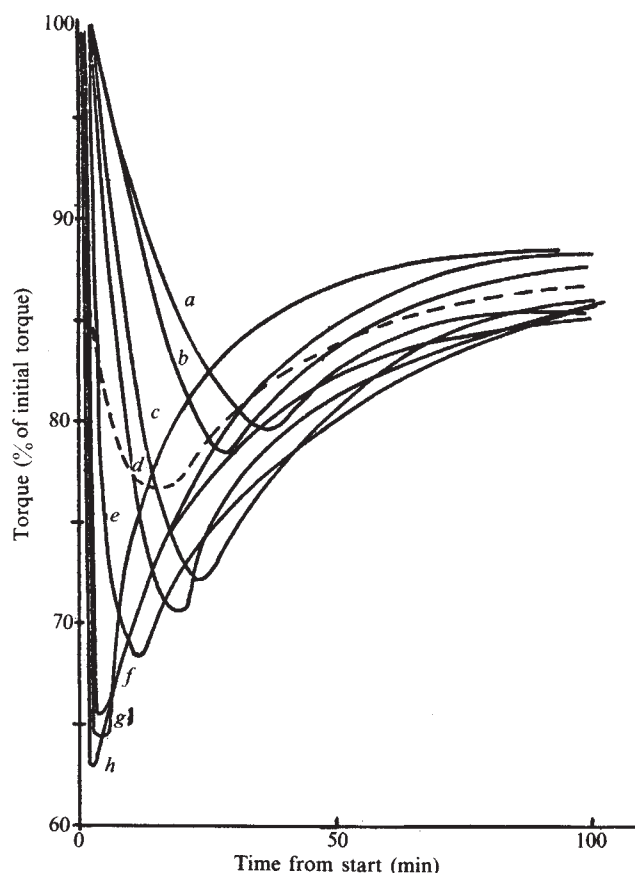


Fig. 2 Torque-time curves obtained with sieved material. The first figures refer to the BS sieve sizes, those in brackets to the mean aperture in millimetres. *a*, below 22 (over 0.71); *b*, 22–25 (0.655); *c*, 25–30 (0.55); *d*, 30–36 (0.46); *e*, 44–85 (0.26); *f*, 85–150 (0.141); *g*, 150–300 (0.079); *h*, over 300 (below 0.053). Polyox Coagulant, 0.86 p.p.m., 840 r.p.m., 17° C.

torque which, after passing through a minimum, gradually approaches an asymptotic value. This is significant because the torque might have been expected to decrease monotonously as the polymer dissolved. Experiments with sieved powder samples (Fig. 2) show that for a given final concentration, the finer the powder used the lower the minimum torque and the shorter the time taken to reach it; the 'steady-state' torque is,

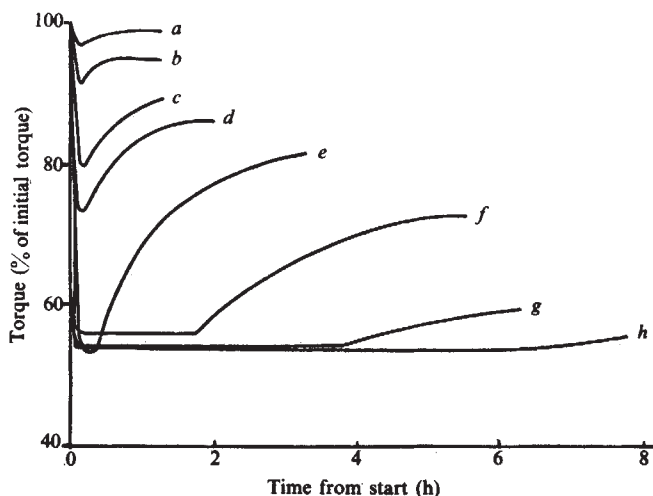


Fig. 3 Effect of polymer concentration (p.p.m.). *a*, 0.086; *b*, 0.171; *c*, 0.43; *d*, 0.86; *e*, 1.71; *f*, 4.30; *g*, 8.60; *h*, 17.1. Polyox Coagulant. 36–44 mesh (0.39 mm), 840 r.p.m., 17° C.

however, unaffected. The effect of polymer concentration using a powder of uniform particle size is shown in Fig. 3. In the particular conditions used the maximum attainable torque reduction was about 50%. This probably corresponds to complete flow laminarisation or some other hydrodynamic limitation, such as Virk's maximum drag reduction asymptote⁶.

On the basis of these observations it is possible to advance the following hypothesis. When PEO powder is added to water, the particles rapidly swell to form a relatively small number of gel-like entities which circulate with the liquid and possibly attach themselves to the disk surface. By the action of shear forces at or near this surface they are broken down into a much larger number of aggregates of entangled molecules. At any one time there will be a particular distribution of aggregate sizes, which will tend to a steady state at long times.

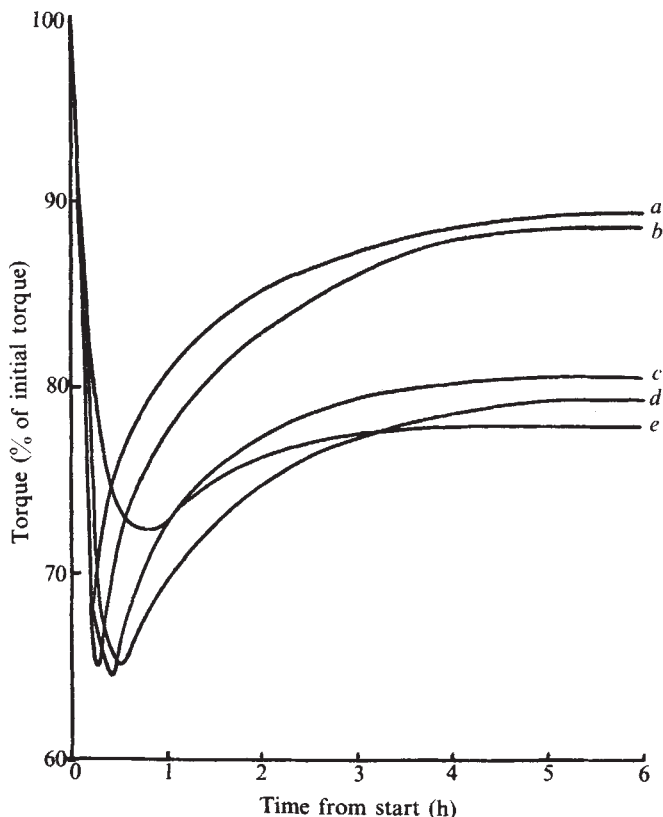


Fig. 4 Effect of disk speed (r.p.m.). a, 840; b, 720; c, 600; d, 420; e, 360. Polyox Coagulant, 30-36 mesh (0.46 mm), 0.86 p.p.m., 17° C.

Thus, the mean aggregate size will decrease monotonously with time while the number of aggregates or free molecules will increase. As the ability of polymer molecule aggregates to interact with eddies and thus bring about drag reduction is presumably a function of aggregate numbers and/or relaxation time, for a given set of hydrodynamic conditions the drag reduction could pass through a maximum as the optimum size distribution was reached and passed.

Strong evidence in favour of the aggregate hypothesis is provided by the effect of rotation rate on drag reduction (Fig. 4). It was found that the long-time value of the drag reduction decreased as the rotation rate increased. This observation runs contrary to the most firmly established fact in the field, that drag reduction in a stable system increases with shear rate, and suggests that the equilibrium (long-time) size distribution depends on the shear history of the solution. To test this idea, the apparatus described was used to shear a solution of PEO for 6 h at 420 r.p.m., after which the torque/

speed characteristic of the disk was measured. This characteristic (Fig. 5) was quite different from that in an identical solution which had been sheared for the same length of time at 720 r.p.m. The solution subjected to the higher shear exhibited less drag reduction, presumably because of the reduced size of the active molecular aggregates.

Although these results could also be explained on the basis of molecular degradation, the effect of molecular weight (Fig. 6) supports the view that cluster formation and breakdown, rather than degradation, is the dominant effect. Thus, for a given polymer concentration, decreasing the molecular weight of the PEO from 4×10^6 to 6×10^5 more than halved both the maximum and steady-state drag reduction, while PEO of molecular weight 1×10^5 gave no drag reduction at all. Although the variation in chain length is only 40:1, the use of the 'entanglement coupling factor'⁶ shows that the likelihood of chain entanglement in the highest molecular weight material is 7,000 times as great as in the lowest.

If the effects observed were a result of degradation, starting materials of different molecular weights would be expected to degrade, after a long time, to the same average molecular weight, that is, the asymptotic value of the drag reduction would be about the same in each case; in fact,

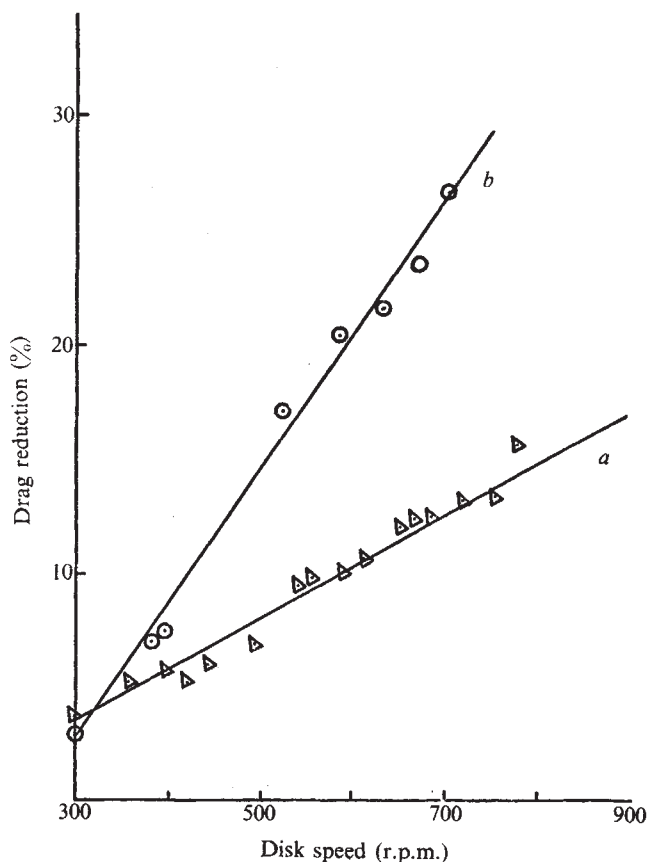


Fig. 5 Drag reduction as a function of speed for solutions sheared at 720 (a) and 420 (b) r.p.m. Polyox Coagulant, 0.86 p.p.m., 17° C.

Polyacrylamide of molecular weight about 5×10^6 exhibited similar drag-reduction characteristics to PEO, but the concentration required was somewhat greater. Guar and locust bean gums behaved differently, the drag reduction increasing with time up to an asymptotic value. The results obtained with PEO and polyacrylamide are consistent with the known tendency of many polymers to form molecular aggregates⁶.

From the standpoint of drag reduction theory, the aggregate hypothesis provides an explanation of many of the anomalies reported in the literature for the PEO-water system. On the

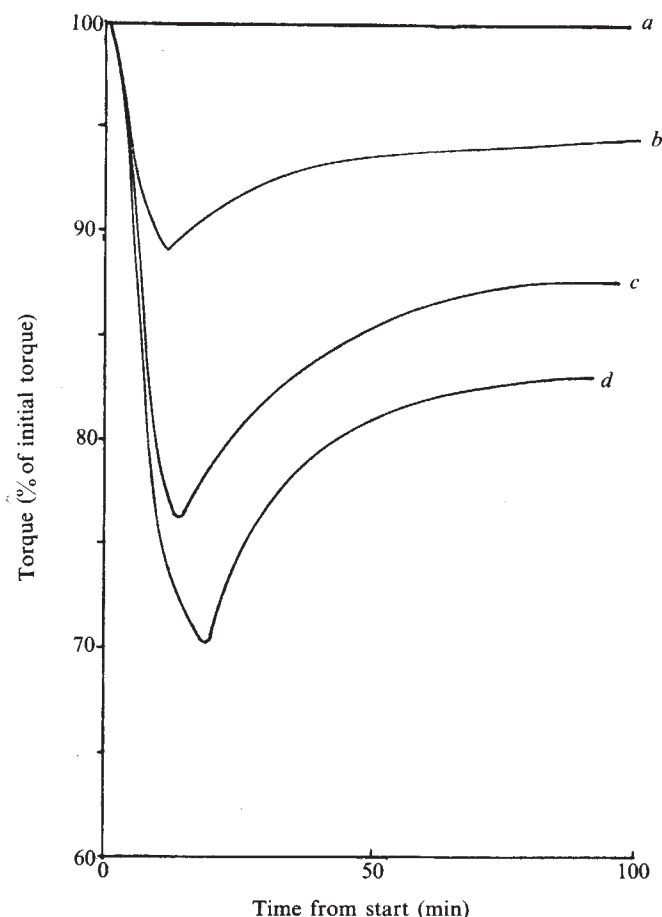


Fig. 6 Effect of molecular weight. Polyox, various grades, 30–36 mesh (0.46 mm), 0.86 p.p.m., 17° C. *a*, WSR N-10 (molecular weight 100,000); *b*, WSR 205 (molecular weight 600,000); *c*, WSR 301 (molecular weight 4,000,000); *d*, Coagulant 1 (molecular weight >5,000,000).

practical side, it suggests possible economies in the use of polymers, particularly PEO, in recirculating and non-recirculating systems; in the former the object should be to stabilise aggregates, in the latter to obtain the correct size aggregates at the right time.

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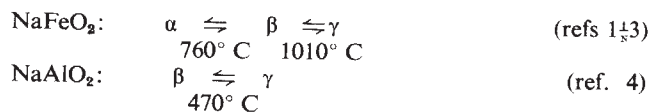
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NaAlO₂ and NaFeO₂ Polymorphism

THE polymorphism of NaAlO₂ and NaFeO₂ is well known. Both oxides form β and γ polymorphs, and although their crystal structures are known, the relationship between the β and γ polymorphs has not been described. This relationship is described here and from it a simple topotactic mechanism for the $\beta \rightleftharpoons \gamma$ transformation is deduced. Movement of only half of the cations is necessary to complete the transformation in either direction and this movement is restricted to a single, filled-empty tetrahedron jump, for each cation. The polymorphisms may be summarised:



An α form may also be prepared at high pressure⁵. Both the β and γ polymorphs have 'tetrahedral structures', that is, the cations and oxygens are all tetrahedrally coordinated. (The α form has an ordered rock-salt structure.)

β -NaFeO₂ and β -NaAlO₂ are isostructural. They have the basic wurtzite structure but with ordering of cations on the tetrahedral sites^{1,2}. This cation ordering causes a lowering of the symmetry from hexagonal P6₃mc in wurtzite to orthorhombic Pbn2₁ in β -NaFeO₂. A schematic (001) projection of the structure (not drawn to scale) is shown in Fig. 1. The unit cell contains two layers of close-packed oxygen atoms at heights 0 and 1/2, and two layers of cations at heights 1/6 and 2/3 (ideally and ignoring differences in cation sizes). The cations for example those at 1/6 are coordinated to three oxygens at 0 and one oxygen at 1/2. Thus, the MO₄ tetrahedra all point upwards, along *z*; linkage of adjacent MO₄ tetrahedra is by corner sharing only (Fig. 2*a*).

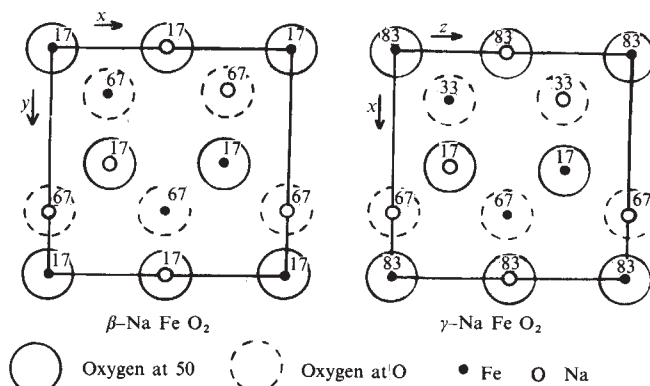


Fig. 1 Schematic projection of β -NaFeO₂ and γ -NaFeO₂ structures. The diagrams are not drawn to scale. Atomic heights, in units of *c*/100 and *b*/100 respectively, are idealised.

The structures of γ -NaAlO₂ and γ -NaFeO₂ have not been determined directly, but they are isostructural with γ -LiAlO₂, tetragonal P4₂12 (ref. 8). The structure of γ -LiAlO₂ was determined independently by X-ray and neutron diffraction on single crystal and powdered specimens, respectively^{7,8}. The structure has been described as a three-dimensional network of linked tetrahedra⁷, its relation to cristobalite, SiO₂, has been noted⁸, and it has been described as a stuffed, distorted cristobalite structure⁸.

I made a model of γ -LiAlO₂ using cardboard tetrahedra and an alternative way of describing its structure became apparent (Figs 1 and 2*b*). Rather buckled layers of oxygen atoms occur, ideally at 0 and 1/2, which again approximate to hexagonal close packing. The cations are distributed over both sets of available tetrahedral sites; that is, at 1/6, 2/3 and

1/3, 5/6, but only half of the sites in each set are occupied (the set of tetrahedral sites at 1/3, 5/6 in β -NaFeO₂ are empty). The cations at for example 1/3 are coordinated to three oxygens at 1/2 and one oxygen at 0 and so these MO₄ tetrahedra point downwards. The manner of linkage of the tetrahedra is different from that in β -NaFeO₂ in that the tetrahedra occur in pairs. Each NaO₄ tetrahedron shares one edge with an adjacent FeO₄ tetrahedron, and *vice versa*. The remaining linkages are by corner sharing.

The β and γ structures may be classified using the nomenclature of Ho and Douglas⁶: ... P_A T₊ P_B T₊ P_A ... for β -NaFeO₂; ... P_A T₊_{1/2} T_{-1/2} P_B T₊_{1/2} T_{-1/2} P_A ... for γ -NaFeO₂. P_A P_B P_A ... refers to anion layers in hexagonal close packing; T₊, T₋ indicate layers of tetrahedral sites which point up and down respectively, and T_{-1/2} indicates that only half of the sites in that layer are occupied.

The structural relationship between β -NaFeO₂ and γ -NaFeO₂ is shown in Fig. 1. The oxygen arrangement is the same in both cases and half of the cations, both Na and Fe, occupy similar positions in the two structures. The remaining Na and Fe cations, however, occupy different tetrahedral sites in the β and γ structures.

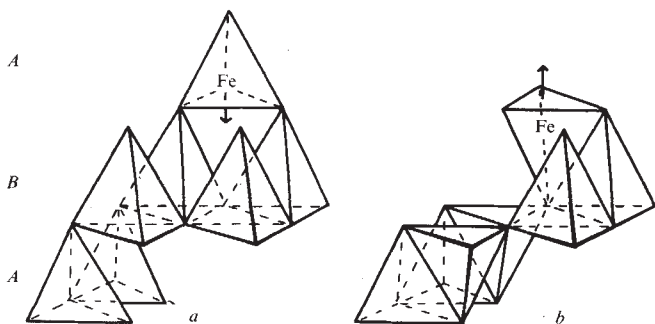


Fig. 2 Schematic structural relationship between β -NaFeO₂(a) and γ -NaFeO₂(b). A, B refer to a hexagonal close-packing sequence.

Closer examination of the structural differences between β and γ forms shows that the $\beta \rightleftharpoons \gamma$ transformation may be effected by a simple topotactic mechanism. Cations at 1/3 in γ -NaFeO₂ can move to 2/3 (as in β -NaFeO₂) simply by jumping through their tetrahedron bases, that is, by passing between the three oxygens at height 1/2 to which they are coordinated. For each cation, the bond to the oxygen at 0 must therefore break and a new bond to the oxygen at 1 will form. Similarly, cations at 5/6 can transform to 1/6 and *vice versa*, by moving through the triangular window of oxygens at height 0. The relationship between the structures, and the mechanism of transformation, are shown in Fig. 2. The β -structure is shown schematically (Fig. 2a), with all of the tetrahedra pointing upwards, as is the corresponding γ structure (Fig. 2b), with half of the tetrahedra pointing upwards and half pointing downwards. The $\gamma \rightarrow \beta$ transformation, written as ... P_A T₊_{1/2} T_{-1/2} P_B T₊_{1/2} T_{-1/2} P_A ... $\rightarrow$... P_A T₊ P_B T₊ P_A ... can be effected simply by inverting those tetrahedra which point downwards (Fig. 2b), and *vice versa* for the $\beta \rightarrow \gamma$ transformation.

Studies on related oxides have revealed several other sets of β , γ type systems. For example, Li₂BeSiO₄ and Li₃PO₄ have low temperature β polymorphs and high temperature γ polymorphs which are related to, but not isostructural with, β and γ -NaFeO₂. Again a close structural relationship exists between the β and γ polymorphs of each pair and a simple topotactic

mechanism can be postulated for the $\beta \rightleftharpoons \gamma$ transformation. These results will be described soon.

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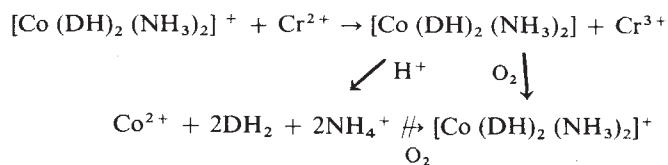
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Cr²⁺-cobaloxime electron transfer reactions

COBALT complexes of dimethylglyoxime (CH₃C:NOH.C:NOH.CH₃), commonly known as cobaloximes, form alkyl derivatives with unusually stable cobalt-carbon bonds^{1,2}. These are regarded by some workers as 'model' compounds for vitamin B₁₂, a biological alkylating agent, the action of which depends on the reversible formation of a similar Co-C bond³⁻⁵. Various mechanisms have been postulated for this reaction⁶. The mechanisms of transalkylation reactions undergone by cobaloximes are thus of great interest; such reactions have been investigated with Hg²⁺ (refs 7 and 8) and with Cr²⁺ (ref. 9). For comparison we are studying the reaction kinetics of other cobaloximes with Cr²⁺. These are apparently electron-transfer reactions¹⁰⁻¹², and should also be considered in the context of the large volume of work that has been done in that field.

Using DH⁻ to represent the mono-anion of dimethylglyoxime, the simple cobaloximes are of the form [Co(DH)₂B₂]⁺, in the case where B is a neutral ligand such as NH₃ or H₂O. Neutral and anionic complexes are well known, with one and two anionic ligands respectively, and the alkyl complexes are usually neutral species of the type [RCo(DH)₂B]. In all cobaloximes the two dimethylglyoximate ions occupy a plane, and are linked by strong hydrogen bonds to form a pseudo-macrocyclic ligand with an N₄ donor set.

We have established that in cases in which both axial ligands are NH₃ molecules, the cobaloxime is reversibly reduced by Cr²⁺:



Cobaloxime(II), the reduced form, was identified by its visible absorption spectrum, and the kinetics of the redox reactions were monitored at 464 nm, where the product absorbs strongly. Cobaloxime(III), regenerated by oxidation of the solution (in air), was isolated as the insoluble perchlorate salt, and microanalysis showed that no chromium was incorporated. In strongly acid solutions (pH < 1), aquation of the reduced species is rapid; this process is acid catalysed and has an equilibrium strongly dependent on [H⁺].

The initial redox reaction obeys the rate law

$$\frac{d[\text{Co}^{\text{II}}]}{dt} = k_{\text{obs}}[\text{Cr}^{2+}][\text{Co}^{\text{III}}],$$

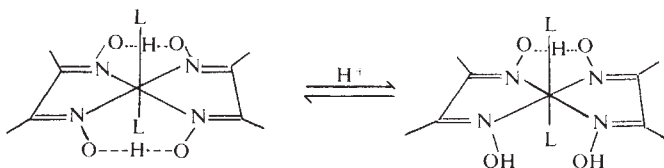
where $k_{\text{obs}} = 40 + 780 [\text{H}^+]$ (at 25°C)

The precise mechanism of this reaction is not fully understood. Although there is no evidence for ligand transfer, nor for a binuclear complex intermediate, an inner-sphere pathway involving the binding of chromium to one or two oxime oxygen atoms cannot be entirely discounted.

The rate depends on $[\text{H}^+]$ because cobaloximes act as weak bases, thus



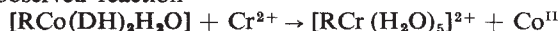
The K_B values for many neutral alkylcobaloximes have been determined^{1,7}, and for these species are generally of the order of 3-4. For the cationic simple cobaloximes a lower value is expected because of the charge effect; our attempts to determine K_B values for the bisammino complex have so far been unsuccessful, but pH measurements in acid solution indicate an upper limit of about 0.5, and the linear dependence of the reaction rate on $[\text{H}^+]$, determined to $[\text{H}^+] = 0.7 \text{ M}$, implies an upper limit of about 0.2. There is now considerable evidence^{13, 14} that this protonation of cobaloximes leads to a significant change in structure:



Weakening of one of the strong hydrogen bonds of the pseudomacrocyclic equatorial ligands allows greater freedom of movement for at least two of the oxime nitrogen atoms relative to the cobalt ion. Thus the Frank-Condon energy barrier to the electron-transfer reaction, which results from the need for changes in coordination geometry on reduction of the metal ion, is greatly lowered. Endicott¹⁵ has suggested that in a similar case, in which the ligand is a true macrocycle, this is the major barrier to redox processes. The reduction of electron density around the cobalt ion when the ligand is protonated should also increase the rate of electron transfer to that ion. The combination of these two effects clearly far outweighs the decrease in stability of the 'precursor' ion pair, or complex, formed by the two cationic reagents, when the charge on the cobalt complex is increased.

When alkylcobaloximes react with Cr^{2+} , the rate also depends on the acid. Both terms in the expression for the rate constant are lower than those that we have observed and the protonated and non-protonated species react at virtually the same rate.

If the cobaloxime is considered as a complex of Co^{III} , then the observed reaction



is an electron-transfer process. As an inner-sphere reaction it would be unique in that the bridging ligand (for example CH_3^-) has no lone pair available to coordinate to the reductant ion while bound to the oxidant. A process can, however, be considered, intermediate between inner and outer sphere, in which alkyl transfer accompanies electron transfer although no bridged precursor complex is formed. Alternatively the alkylcobaloxime can be regarded as a complex of Co^{II} , in which case the most plausible reaction mechanism is a bimolecular homolytic substitution at the carbon atom, an $\text{S}_{\text{H}2}$ process. Both of these mechanisms are considered to be possible⁹.

The absence of a rate increase on protonation of the alkylcobaloxime is however, inconsistent with the postulate of an electron-transfer process in this case, but it indicates a process in which the electron density at the Co ion is not greatly changed. (In this context it should be noted that only the

non-protonated alkylcobaloximes react with Hg^{2+} (ref. 7). Consideration of the charge effect indicates that this is consistent with the formulation of that reaction as a net oxidative process on cobalt, although it may be expected that the diminution of the Frank-Condon barrier would be the dominant effect in such a reaction. It is generally agreed^{7,8} that this is an electrophilic substitution reaction at carbon, an $\text{S}_{\text{E}2}$ process.)

Further insight may be obtained from a consideration of the redox potentials of the reagents involved. These have been determined for both simple¹⁶ and alkylcobaloximes^{17,18}. Schrauzer and Windgassen¹⁷ report one-electron reduction potentials for $[\text{MeCo}(\text{DH})_2\text{H}_2\text{O}]$ of -1.7 and -2.42 V in acetonitrile against Ag/AgNO_3 (0.1 M), compared with -0.67 -1.44 and -2.42 for $[\text{Co}(\text{DH})_2(\text{Py})_2]^+$ under the same conditions. They conclude that the alkyl species are complexes of Co^{II} , and report¹⁴ that the product of the first reduction is a Co^{I} species¹⁹. Honokabe and Yamazaki¹⁸ seem to attribute the first cathodic wave to a $\text{Co}^{\text{III}}-\text{Co}^{\text{I}}$ reduction, although they discuss it subsequently in terms of a one-electron process. Their results do however agree on the value of the first half-wave potential, when corrected for different reference electrodes. According to Maki¹⁶, the first half-wave potential of the bisamminocobaloxime is only 0.43 V more negative than that of the bipyridine complex. The methylquo species thus has a reduction potential 0.6 V more negative than the bisammine complex. In the latter case, the reduction is reversible¹⁶, and can therefore be corrected for the reference electrode to give a value for the standard electrode potential²⁰ of -0.37 V . Because E^0 for the $\text{Cr}^{2+}/\text{Cr}^{3+}$ couple is -0.41 V ²¹, the reduction by Cr^{2+} of any species with a reduction potential much more negative than that of the bisamminocobaloxime is, thermodynamically, an unfavourable process. The electron-transfer process by which that complex reacts with the chromous ion is therefore unavailable to the alkyl complexes.

We therefore conclude that it would be incorrect to regard the reaction of Cr^{2+} with alkylcobaloximes as a chromium-cobalt electron transfer reaction. The simplest mechanism consistent with all of the described observations is homolytic substitution at the carbon atom.

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BIOLOGICAL SCIENCES

Exciton-like splitting in acridine dye-nucleic acid complexes

COMPLEXES between cationic acridine dyes and nucleic acids are of considerable interest because of the wide variety of biological effects in which the interaction between the dye and nucleic acids have been implicated¹⁻³.

Lerman's evidence based on the X-ray study and hydrodynamic measurements of proflavine-DNA complexes remains the most persuasive for the intercalation model, that is the mass per unit contour length of DNA diminishes when complexed with the dye⁴⁻⁶. Most recent information about the nature of complexes in solution derives from studies of equilibria between free and bound dye in the form of binding curves¹ (Scatchard plots).

In spite of several studies of complexes between the chromosomal stain quinacrine (QAC)⁷⁻¹³, and nucleic acids, the molecular nature of the complex is not understood¹⁴⁻¹⁷. We have now used ultraviolet absorption spectroscopy to look for exciton-like interaction in complexes between QAC and DNA, poly(dAT), and poly(dG)·poly(dC). We also measured rate constants for quenching of the fluorescence of the complexed dye by iodide ion.

The interaction of excited states of weakly coupled composite systems such as molecular crystals, dimers, aggregates and polynucleotides can be treated by the molecular exciton model¹⁸. The interaction between dyes and nucleic acids when the binding is strong (at high phosphate:dye (P:D) ratios) can be treated similarly¹⁹. In the simple theory, when only the transition dipole-dipole potential term is considered, significant interaction occurs if (a) there is a small energy difference between interacting states, and (b) the interacting transition dipoles have large transition moments.

On the basis of the intensity and energy of the transition, the S_0 absorption band of QAC (pH 7) ($35,710\text{ cm}^{-1}$ (280 nm) $f = 1.15$) is the only band which could possibly show significant exciton-like interaction (in addition to dispersion and other solvent shifts) with the polynucleotide transition at $38,460\text{ cm}^{-1}$ (260 nm, $f = 0.10$). The other two bands, S_1 at $22,470\text{ cm}^{-1}$ (445 nm, $f = 0.18$) and S_2 at $29,070\text{ cm}^{-1}$ (344 nm, $f = 0.08$) ought to show dispersion and possibly other shifts in the complexes but not exciton-like interaction.

To ensure complete, strong primary binding¹, we worked with P:D ratios of at least 6:1 and usually 20:1. Consequently the strong 260 nm absorption in the spectrum of the polynucleotide which was unperturbed by bound dye, masked small changes in the QAC S_0 transition. Therefore, we recorded the difference spectra of dye-polynucleotide solutions (sample beam) against identical polynucleotide samples (reference beam) on our Cary 14 spectrophotometer.

Figure 1 shows spectra of free QAC and difference spectra of QAC bound to DNA, poly(dAT) and poly(dG)·poly(dC). In the spectrum of QAC bound to DNA the 445 nm and 344 nm bands of free QAC appeared at 455 nm ($21,980\text{ cm}^{-1}$) and 350 nm ($28,570\text{ cm}^{-1}$) respectively. These transitions showed dispersion shifts only. In the 260-300 nm region, however, two overlapping bands were observed at 290 nm ($34,480\text{ cm}^{-1}$) and 270 nm ($37,040\text{ cm}^{-1}$). If these two bands are decomposed into their components, we obtain two bands with maxima at $34,480\text{ cm}^{-1}$ and $37,540\text{ cm}^{-1}$. The energy difference between these peaks (Δ) is 3060 cm^{-1} . Thus Δ is larger than the energy difference between the unperturbed interacting states, ΔE_{31} ($38,460 - 35,710 = 2,750\text{ cm}^{-1}$) by 310 cm^{-1} .

Relative to an area of 1.15 for the oscillator strength of the $S_0 \rightarrow S_1$ transition of the free dye, we observed areas of 0.40 and 0.30 for the 290 nm and 270 nm components,

respectively, for the spectrum of the complex. A similar band pattern is observed with the QAC/poly(dAT) complex (Fig. 1b). Decomposing the two overlapping bands as before we obtained two bands at $37,315\text{ cm}^{-1}$ and $34,015\text{ cm}^{-1}$, giving $\Delta = 3,300\text{ cm}^{-1}$, again larger (by 550 cm^{-1}) than $\Delta E_{31} = 2,750\text{ cm}^{-1}$.

In contrast to the results with DNA and poly(dAT), the difference spectrum of QAC bound to poly(dG)·poly(dC) (Fig. 1c) contained only a single broad band in the region of the 280 nm absorption of the spectrum of free QAC.

No noticeable change in the difference spectra was observed when the P:D ratio was varied from 50:1 to 5:1. However, when the P:D ratio was reduced from 5:1 to 1:1, the doublet became a broadened maximum at 280 nm. Since the low P:D region corresponds to that in which weak binding occurs²⁰, the absorption at 280 nm can be attributed to weakly bound or free dye.

The dramatic difference in the 280 nm region of the spectra of free QAC bound to DNA and poly(dAT) is good evidence that the interaction between the S_0 state of QAC and its environment is not due to a simple dispersion shift.

Since the interaction energy (310 cm^{-1}) is smaller than the initial level separation ($2,750\text{ cm}^{-1}$) by almost an order of magnitude, the exciton model is not strictly applicable either. However, the transition moments are associated with broad bands rather than sharp lines and these bands overlap considerably (see Fig. 1). Therefore, resonance type interactions do occur as in Förster energy transfer and this may account for the exciton-like behaviour, that is the large intensity redistribution and pronounced band shifts and splitting. It therefore seems reasonable to attribute the two

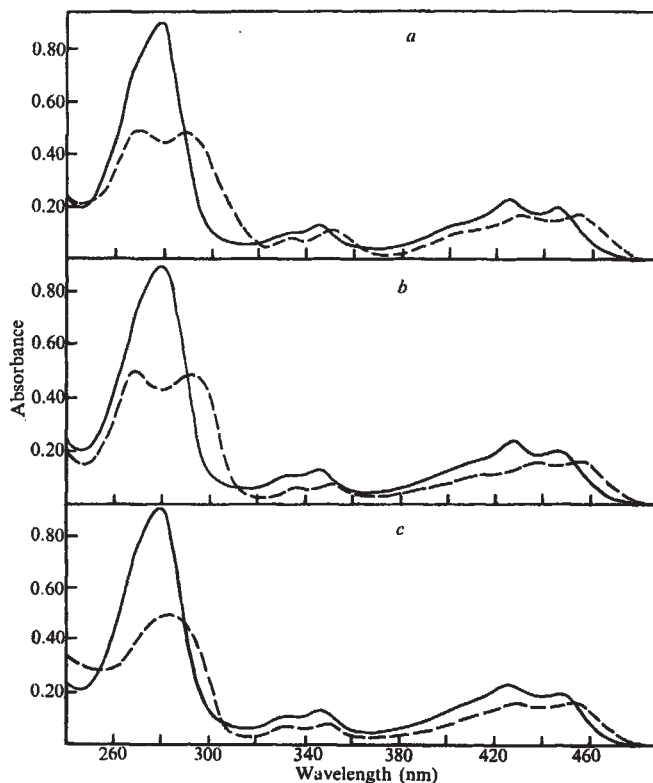


FIG. 1 Quinacrine-polynucleotide difference spectra. —, Free quinacrine $1.55 \times 10^{-5}\text{ M}$; ---, quinacrine $1.55 \times 10^{-5}\text{ M}$ and polynucleotide $3.1 \times 10^{-4}\text{ M}$. a, Calf thymus DNA; b, poly(dAT); c, poly(dG)·poly(dC). Difference spectra were run on a Cary 14 spectrophotometer (dispersion = 20 Å mm^{-1}) using 1 cm cells at 23°C . The reference cell contained a concentration of polynucleotide equal to that in the sample cell which contained the quinacrine-polynucleotide solution. Identical maxima were observed for reference optical densities of 0.6 (slits, 1 mm) and 1.85 (slits, 2.5 mm).

TABLE 1 Fluorescence quenching of quinacrine and quinacrine/nucleic acid complexes by iodide ion

	Φ_F^f/Φ_F^{f+b}	τ , ns	$k_q \times 10^{-9}$ corrected	$M^{-1} s^{-1}$ uncorrected
QAC	1	4	7.8	—
QAC + poly(dAT)	0.4	20	0.19	0.20
QAC + poly(dG)				
poly(dC)	7	6	2.3	3.0
QAC + DNA	2.1	10	1.1	1.2

[QAC] = 2×10^{-5} M; P:D = 20:1. The solution was buffered at pH 7 with 0.01 M cacodylate. The ionic strength was maintained constant at 0.1 M by the addition of appropriate amounts of NaCl such that $[Cl^-] + [I^-] = 0.1$ M. The polynucleotides were purified by dialysis for 24 h. Calf thymus DNA was purchased from Worthington Biochemicals; poly(dG)·poly(dC) and poly(dAT) were purchased from Miles Laboratories. Fluorescence intensities were measured using a Farrand spectrofluorimeter exciting at 365 nm (bandwidth 5 nm) and monitoring at 490 nm. Lifetimes were measured by the TRW model 75A fluorescence lifetime apparatus. Rate constants were obtained from Stern-Volmer plots²² of fluorescence intensity compared with [iodide]. Φ_F^f/Φ_F^{f+b} (=A) is the ratio of the quantum yield of the free dye to that of free + bound dye. The corrected rate constants were derived from values of I_b^0/I_b obtained according to²²: $I_b^0/I_b = [1 - (1 - \alpha)A]/[(I_f^0/I_b + I_f^0/I_b) - (1 - \alpha)A(I_f^0/I_b)]$ where I is the fluorescence intensity, the superscript zero designates the fluorescence at zero iodide concentration; the subscripts f , b , $f + b$ designate free dye, bound dye, and free plus bound dye respectively; α is the fraction of bound dye (0.95). I_f^0/I_b are the observed values of the fluorescence intensity at various iodide concentrations.

maxima which we have observed from the difference spectra as being due to two states formed from the dipole-dipole interaction between the S_2 state of QAC and the S_1 state of DNA with intensity being stolen from the intense $S_0 \rightarrow S_2$ transition of QAC. In the case of dipole-dipole interactions the interaction energy varies as r^{-3} . We conclude therefore that the binding of QAC to poly(dG)·poly(dC) and to the other two polynucleotides is such that the distance of approach between QAC and the G-C base pairs is much larger than that between QAC and the A-T base pairs. In this sense, the observed splitting in DNA and poly(dAT) and the limiting P:D ratio of 5 at which the splitting is observed is fully consistent with the intercalation model.

Since the more tightly wound G-C helix is less likely to unwind than the other two polynucleotides in order to accommodate the intercalating dye molecules, the different difference spectra observed for poly(dAT) and DNA dye complexes on the one hand, and the poly(dG)·poly(dC)-dye complex on the other supports the intercalation model in the former two polynucleotides.

The constant shape of the Franck-Condon envelope of the difference spectra when the P:D ratio was varied from 50:1 to 5:1 demonstrates that the observed splitting results from highly localised interactions between dye molecules and their nearest neighbour. There is no evidence for dye-dye interactions in QAC-nucleic acid complexes.

This conclusion suggests that the dye is more sterically hindered, and hence more shielded from collisions with molecules in solution, when bound to poly(dAT) than when bound to poly(dG)·poly(dC). A test for this was to examine the quenching of the fluorescence of the complexed QAC molecule by species such as iodide ions—a collisional process²¹. We would expect differences in the fluorescence quenching rate constant (k_q) for QAC-poly(dAT) and QAC-poly(dG)·poly(dC) complexes. Fluorescence intensity values for quenching²² are less physically significant than k_q , for they do not account for the significant variation in fluorescence lifetime of the dye in the different environments (Table 1).

In the three cases studied, Stern-Volmer²³ plots were linear. Table 1 shows a significant variation in k_q depending on the complex. We applied a correction²² to the observed

k_q values in order to ensure that the differences observed were not due to a small amount of free dye (estimated to be less than 5% from absorbance changes at 440 nm, compared with P:D) which may still be present even at high P:D ratios. There was no significant difference between the corrected and uncorrected values.

The value of k_q for the free dye agrees with the calculated diffusion controlled value in water²⁴. The poly(dAT)-QAC complex had the lowest k_q and poly(dG)·poly(dC)-QAC complex the highest. These observations confirm our interpretation of the spectroscopic experiments described above; that QAC is bound considerably closer (intercalated) to A-T than to G-C base pairs in the polynucleotides.

In summary, (1) we have observed exciton-like splitting between the S_2 state of QAC and the lowest excited state of DNA and poly(dAT) with concomitant intensity redistribution but have not observed this effect with QAC and poly(dG)·poly(dC); (2) we conclude that QAC is bound closer to A-T base pairs (possibly by intercalation) than to G-C base pairs in polynucleotides; (3) the most important interactions responsible for the splitting are nearest neighbour interactions; (4) dye-dye interactions are negligible in QAC-nucleic acid complexes.

Coupled with theoretical calculations we believe that the exciton-like interaction could be quite important in unravelling the structures of acridine dye-nucleic acid and similar complexes as they occur in solution.

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α -Hydrazino-ornithine blocks net synthesis of putrescine but not of RNA and DNA

THE polyamines spermine, spermidine and their precursor putrescine occur ubiquitously and in high concentrations in animals and plants¹. Increased polyamine synthesis in animal cells and bacteria precedes or coincides with enhanced RNA formation, especially during growth stimulation, suggesting a close relationship². Ornithine decarboxylase (ODC), a rate-limiting enzyme in polyamine synthesis, has an extremely short half life³. The early, large increase in its activity in all cases of rapid tissue growth examined suggests that the formation of its product, putrescine, triggers early enhancement of RNA synthesis during cellular growth. A critical test of this concept would be to block net putrescine and (or) polyamine synthesis selectively and determine whether nucleic acid synthesis is altered. Such a test might assess the role of putrescine as a potential modulator of growth in normal and neoplastic tissue.

α -Hydrazino-ornithine (HO) is a specific and potent inhibitor of ODC with K_i values of 0.5 μ M and 2 μ M for bacterial and mammalian enzymes respectively^{4,5}. HO inhibits competitively with both the substrate ornithine and the coenzyme pyridoxal phosphate. HO is 100 times more potent an inhibitor of ODC than are other hydrazines and inhibits the pyridoxal phosphate-dependent glutamic acid and dihydroxyphenylalanine decarboxylases only 1% as efficiently as it inhibits ODC^{4,5}, indicating considerable selectivity for ODC. HO is relatively nontoxic, producing no gross behavioural abnormalities in rats in doses up to 2 g kg⁻¹ (ref. 5). We now report that HO effectively blocks net putrescine synthesis in rat hepatoma cells (HTC) in

TABLE 1 Effect of α -hydrazino-ornithine on DNA and RNA synthesis in rat tissues

Addition	DNA (c.p.m.) in HTC cells	RNA (c.p.m.)	
		Intact liver	Regenerating liver
None	19,700 $\pm$ 1,500 (n = 3)	1,290 $\pm$ 140 (n = 4)	1,610 $\pm$ 70 (n = 4)
α -Hydrazino-ornithine	20,600 $\pm$ 1,200 (n = 3)	1,200 $\pm$ 90 (n = 5)	2,250 $\pm$ 140 (n = 5)

HTC cells were collected and stimulated by dilution (1:5) with fresh growth medium in the presence and absence of α -hydrazino-ornithine (5×10^{-4} M). At 17 h, ³H-thymidine (1 μ Ci ml⁻¹; 6–7 Ci mmol⁻¹) was added and incorporation into 5% trichloroacetic acid (TCA)-precipitable material was measured in 1 ml aliquots of cells (25 μ g protein) after a 1 h pulse at 37°C (methods as in legend to Fig. 1). DNA incorporation is expressed as c.p.m. $\pm$ s.e.m. per sample. The incorporation of ¹⁴C-orotic acid (6.6 mCi mmol⁻¹) into rat liver was measured in 5% TCA-precipitable material 10 min after intravenous administration of 1 μ Ci of orotic-6-¹⁴C-acid¹³. α -Hydrazino-ornithine (200 mg kg⁻¹) was given subcutaneously to sham operated and hepatectomised animals at operation and 4 h later; animals were killed 7 h after hepatectomy. Hepatic tissue was excised, quick frozen, weighed and homogenised in 9 volumes of 0.32 M sucrose. Aliquots (0.5 ml) were precipitated by adding 0.3 ml of 20% TCA, and the precipitate was washed with four successive 4-ml portions of 5% TCA and two 4-ml portions of absolute methanol. The resulting precipitate was dissolved in 1 ml of hydroxide of hyamine at 70°C for 30 min and transferred with 1 ml of absolute ethanol to 10 ml of PCS solubiliser for scintillation counting. RNA incorporation is expressed as c.p.m. $\pm$ s.e.m. per sample (0.057 g wet weight of tissue).

culture and in the liver of intact rats. Doses of the drug which markedly reduce putrescine do not appreciably affect the synthesis of either RNA or DNA.

The growth stimulus produced when high density HTC cells were diluted with fresh medium led to a marked increase in putrescine (Fig. 1). This increase followed soon

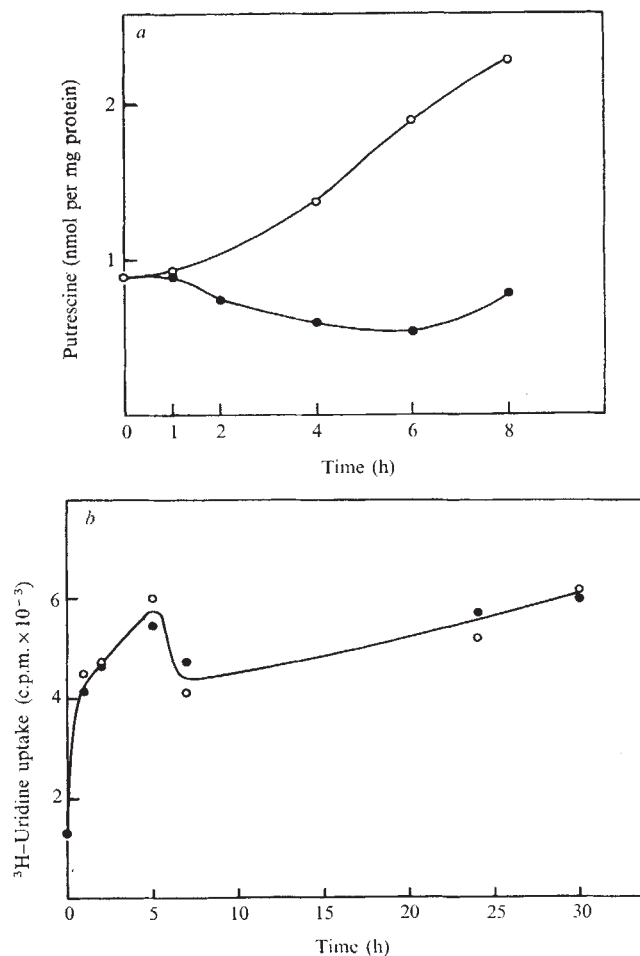


FIG. 1 Influence of α -hydrazino-ornithine on putrescine levels (a) and RNA synthesis (b) in HTC cells. a, HTC cells were grown to high density (about 2×10^7 per flask) in monolayer in 75 cm² Falcon flasks as described previously⁸. Cells were collected by scraping, and diluted (final concentration about 2×10^5 ml⁻¹) with fresh medium (10% foetal calf serum in Eagle's minimum essential medium with Hanks balanced salts, supplemented with 100 U each of penicillin and streptomycin, and equilibrated with 5% CO₂ in air). Putrescine was analysed by an enzymatic-isotopic microassay¹⁴ in cell extracts. Aliquots (approximately 2.5×10^6 cells) were collected by centrifugation, washed twice at 4° with 10 ml phosphate-buffered saline, and disrupted by freezing and thawing four times in 0.25 ml distilled water. Protein was precipitated by heating at 95° C for 15 min and putrescine was assayed in the supernatant fluid. Results are expressed in nmol putrescine per mg cell protein, determined on aliquots of cell lysates by the method of Lowry *et al.*¹⁵ b, High-density HTC cells collected by scraping were diluted 1:5 with fresh medium and incubated at 37° C in 1 ml aliquots (final concentration 2×10^5 cells per ml). ³H-uridine (1 μ Ci ml⁻¹; 26 Ci mmol⁻¹) incorporation was measured by a 1 h pulse at timed intervals after dilution. Cells were washed by centrifugation at 4° C with three successive 5 ml aliquots of Hanks buffer, 5% TCA and absolute methanol. The final precipitate was solubilised in 0.5 ml of hyamine in methanol at 70° C for 15 min, and the solution was mixed with 3 ml of absolute ethanol for scintillation counting. Uridine uptake is expressed in c.p.m. incorporation per sample (average of triplicate samples). ○, Cells without α -hydrazino-ornithine; ●, cells with 5×10^{-4} M α -hydrazino-ornithine.

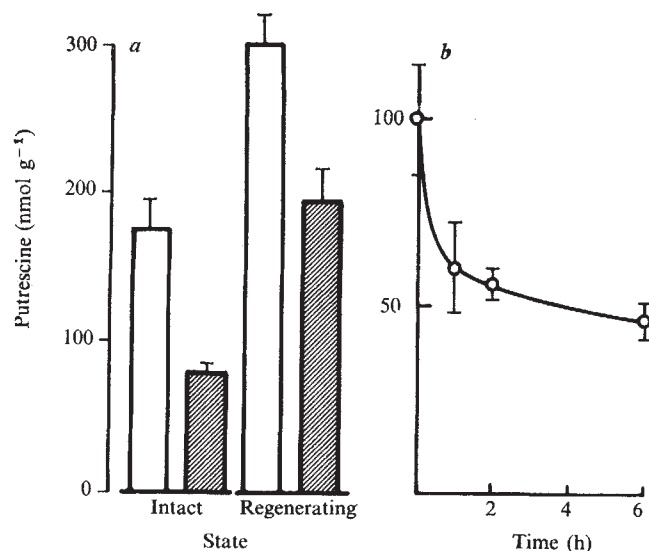


Fig. 2 Effects of α -hydrazino-ornithine on putrescine levels in intact and regenerating liver. *a*, Male rats (130 g, groups of three) under ether anaesthesia were nephrectomised bilaterally, with or without concomitant partial hepatectomy. Four hours after operation, animals received either saline (0.9% NaCl) or α -hydrazino-ornithine (75 mg kg⁻¹, subcutaneous), and were killed 2 h later. Livers were quick-frozen and tissue was homogenised in 9 volumes of distilled water. Protein was precipitated by heating for 15 min at 95° C and putrescine was measured in the supernatant¹⁴. Results are expressed in nmol of putrescine per g wet weight of tissue. Bars above the columns indicate s.e.m. *b*, Intact rats were killed at timed intervals after the subcutaneous administration of 200 mg kg⁻¹ α -hydrazino-ornithine, and hepatic tissue was analysed for putrescine as outlined above. Results are expressed as nmol per g wet wt with vertical bars indicating 2 s.e.m. values. Open columns, saline; hatched columns, α -hydrazino-ornithine.

after that of ODC activity, which reached a maximum after 4–6 h and then declined^{6–8}. In the presence of HO, putrescine levels not only stopped increasing, but they declined. In these cultures ODC activity is completely inhibited, although dialysis of tissue extracts to remove HO unmasks an enhancement of ODC levels⁸. The time course of incorporation of ³H-uridine into high-density monolayer HTC cells diluted with fresh growth medium (Fig. 1) resembles that reported earlier for synchronised HTC cells in suspension⁹. It is interesting that the synthesis of RNA, as reflected by uridine incorporation, was virtually unaffected when putrescine levels were substantially lowered by HO. ³H-uridine incorporation was unaltered in the presence of HO for periods up to 30 h. Because we did not measure specific activities of nucleotide triphosphate pools, changes in precursor uptake may have masked effects of HO on nucleic acid synthesis.

In agreement with previous studies on synchronised HTC cells in suspension⁹, the high-density monolayer cells exhibited maximal ³H-thymidine incorporation 18–20 h after stimulation by dilution with fresh growth medium. At this time there was no appreciable difference between cells stimulated in the absence or presence of HO (Table 1).

In intact animals HO depressed hepatic putrescine levels about 50%, an effect which persisted for at least 6 h (Fig. 2). In regenerating liver, HO prevented the 1.7-fold increase in putrescine usually elicited by partial hepatectomy^{10,11} (Fig. 2). Hepatic putrescine levels were higher in controls, nephrectomised to impair HO excretion and permit administering lower doses, (Fig. 2, left) than in unoperated controls (Fig. 2, right), which is consistent with reports that stress increases hepatic ornithine decarboxylase activity¹². In agreement with earlier data¹³, partial hepatectomy caused a 25–50% increase in liver RNA synthesis,

which is the same in nephrectomised and normal rats. In contrast to its influence on putrescine, HO (50 mg kg⁻¹ to nephrectomised rats; 400 mg kg⁻¹ to nonnephrectomised rats) failed to alter the apparent increase in RNA synthesis (Table 1).

Thus both in HTC cells in culture and in regenerating and intact rat liver, HO effectively blocks net putrescine formation. Neither RNA nor DNA synthesis is apparently altered in HTC cells, and RNA synthesis is unaffected in regenerating and intact liver. HO only partially lowered putrescine levels in our experiments. Conceivably, complete depletion of putrescine might affect nucleic acid formation. Moreover, more refined techniques may reveal influences of HO on the synthesis of certain classes of RNA or DNA. Although we did not measure spermidine and spermine, it is conceivable that their continued synthesis in the presence of HO could mediate enhanced nucleic acid synthesis. However, blockade of spermidine and spermine formation by methyl glyoxal *bis* (guanyldiazotane), which inhibits S-adenosylmethionine decarboxylase, fails to impair nucleic acid synthesis^{16,17}.

Our data indicate that enhanced ODC activity and putrescine formation are not an indispensable signal for new RNA and consequent DNA synthesis. Although increased levels of putrescine and presumably of polyamine formation may not be causally related to new RNA synthesis, the accumulated evidence^{1,2} still suggests that the polyamines serve an important function in tissue growth.

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Hormonal regulation of presumptive mRNA in the fungus *Achlya ambisexualis*

THE Oomycete fungus, *Achlya ambisexualis*, is one of the most primitive eukaryotes which differentiates in response to a steroid hormone¹. Because it is fast growing, easy to manipulate and can be induced to undergo differentiation in a relatively synchronous manner (the entire thallus acts as target cells), *Achlya* lends itself to studies of the molecular control of gene activation by hormones.

Antheridiol, a sterol secreted by female strains of *Achlya* has been characterised from culture filtrates and synthesised from disnorcholenic acid¹. The hormone is active at 6×10^{-12} g⁻¹ when assayed with *A. ambisexualis* (strain E87)¹. Addition

of antheridiol to vegetative hyphae elicits the initiation of antheridial branches² which eventually differentiate into the male sex organs (antheridia). Thomas and Mullins³ postulate that a prerequisite for hormonal induction of antheridial hyphae is a localised softening of the cell wall by cellulase. They suggest that this weakens areas of the walls, through which protoplasm is forced by the internal turgor pressure forming the sex organ initial. Their preliminary studies suggest that both transcription and translation are required for this process⁴. In animals steroid hormones can stimulate transcription of specific mRNAs which are then translated into proteins required for cellular differentiation⁵⁻⁷.

Recent studies have shown that mRNAs of higher plants and animals contain a sequence of polyadenylic acid (poly(A)) which is added to the 3' ends of the molecules⁸⁻¹⁰. Only histone mRNAs lack a poly(A) moiety¹¹. We have demonstrated in *Achlya* the presence of a poly(A)-rich class of cellular RNA. This is the most primitive eukaryote to date in which poly(A) has been shown to be associated with presumptive mRNA. Furthermore, we have demonstrated that the sex hormone, antheridiol, has a considerable effect on the accumulation of this RNA during the early stages of male sex organ development. We have characterised the poly(A)-rich fraction and have demonstrated its similarity to the mRNAs of higher eukaryotes.

Poly(A)-rich RNA has been demonstrated in the cellular slime mould, *Dictyostelium discoideum*¹² and in the yeast, *Saccharomyces cerevisiae*^{13,14}. The size of this poly(A) varies from 50 nucleotides in yeast¹³ to more than 200 nucleotides in higher animals¹⁵⁻¹⁷. In contrast, *Escherichia coli* mRNA, so far as is known, does not contain any poly(A)¹⁸. Two interesting questions are (i) do all existing eukaryotes possess some poly(A) associated with their mRNA, and (ii) does the size of such poly(A) increase with phylogenetic complexity? We have found the size of the *Achlya* poly(A) moiety to be smaller than the poly(A) tracts found in animals and *D. discoideum* but similar to that of *S. cerevisiae*.

Stock cultures of *A. ambisexualis* were maintained on Emerson's YPSS agar (Difco). Asexual spores were prepared according to Griffin¹⁹. A suspension of 1×10^5 spore cysts was inoculated into PYG broth (300 ml)¹⁹ and grown for 24 h at 23° C until a finely suspended mycelium had grown. This was stored at 5° C or used immediately as an inoculum. A 25 ml sample of this was transferred to flasks containing 275 ml of Barksdale's mating media²⁰ and was grown for 24-32 h at 23° C. Cultures were collected through four layers of cheesecloth followed by washing with four volumes of ice-cold deionised H₂O.

RNA was isolated at 5° C by suspending 10-20 g wet weight of mycelia in SSC (0.15 M NaCl, 15 mM Na-citrate, pH 8.3) 1% SLS (Na-lauryl sulphate) and an equal volume

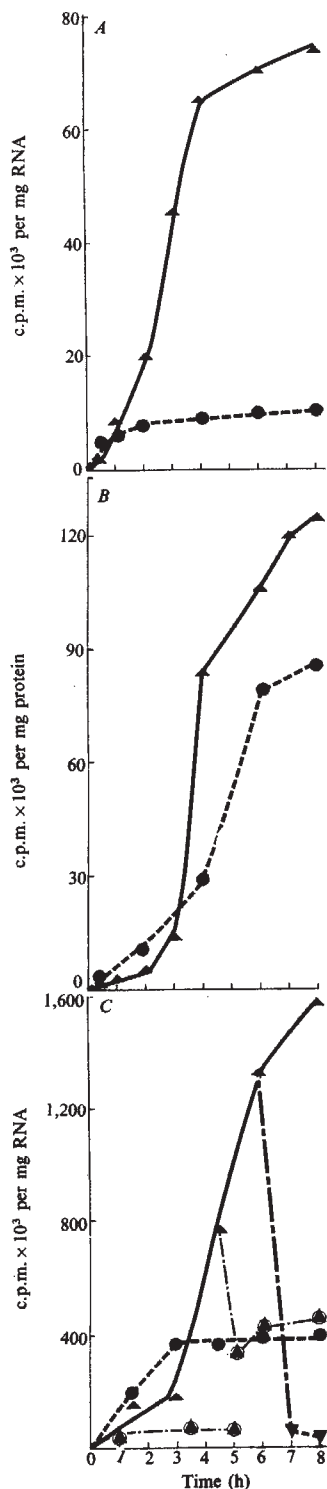


Fig. 1 The effect of antheridiol on the accumulation of RNA and protein during male organ initiation in *A. ambisexualis*. Total phenol-extractable RNA. Cells were exposed to continuous labelling conditions ($10 \mu\text{Ci } ^3\text{H}$ -uridine, specific activity 52 Ci mmol^{-1}) from time zero in the presence (Δ) and absence ($\bullet$) of $5 \times 10^{-11} \text{ g ml}^{-1}$ antheridiol. Concentration of RNA was determined by A_{260}/A_{280} ratios. Radioactivity was determined on a liquid scintillation counter. B, Total hot TCA-precipitated protein. Cells were labelled using steady state conditions ($25 \mu\text{Ci ml}^{-1}$ ^3H -leucine, specific activity 54 Ci mmol^{-1}) in the presence and absence of hormone. Concentration of protein determined by A_{260}/A_{280} ratios or by the Lowry procedure. C, Poly(A)-rich RNA accumulation. Cells were labelled using steady state conditions ($10 \mu\text{Ci ml}^{-1}$) in the presence (Δ) and absence ($\bullet$) of hormone. Total RNA was extracted as described in the text and passed through a Sigma 38 cellulose column (Fig. 2). RNA concentration and radioactivity were determined as described above. When indicated $10 \mu\text{Ci ml}^{-1}$ actinomycin D (---) (ref. 19) $25 \mu\text{g ml}^{-1}$ cordycepin (—) (ref. 9) was added to the hormone-treated cultures.

TABLE 1 Base composition analysis of *A. ambisexualis* RNA from unmodified cellulose columns

RNA fraction	Base composition (%)			
	C	G	A	U
+ Hormone (bound)	17.3	21.5	33.5	27.5
Vegetative control (bound)	16.6	20.5	34.5	27.8
Unbound (both hormone and control)	17.4	23.1	29.4	29.9

Phosphorus-32 ($15 \mu\text{Ci ml}^{-1}$, carrier free)-labelled RNA fractions were collected from Sigma 38 cellulose columns (Fig. 2) made up to SSC, 1% SLS, 0.3 M LiCl and precipitated in 2.5 volumes of ethanol. The precipitate was taken up in 0.3 N KOH and hydrolysed at 37°C for 24 h. Base composition was determined using descending paper chromatography following the procedure of Lane²⁵.

of phenol, equilibrated with 50 mM Tris-HCl, pH 8.3. The mixture was then homogenised in a Willems polytron homogeniser at the highest setting for 10 s. The aqueous phase was extracted three times with the Tris-washed phenol. This phase was made 0.3 M with respect to LiCl and the precipitated RNA was washed twice in ethanol, dried and dissolved in deionised H_2O .

To prepare partially purified nuclei, 30–50 g wet weight of tissue was suspended in SSC buffer containing 1% SLS, and 0.25 M sucrose according to the procedure of Jaworski and Horgen²¹. The nuclear pellet (containing some mitochondria) was resuspended in SSC, 1% SLS and extracted with phenol, precipitated with ethanol and taken up in deionised H_2O as described above. Polyribosomes were isolated according to the method of Lin and Key²², except that buffers were at pH 9.0. Polysome fractions were pooled and the RNA was extracted as described.

In the presence of 5×10^{-11} g ml^{-1} antheridiol at 23°C , antheridial branch initials are visible between 3 and 4 h after the addition of hormone. The incorporation of ^3H -uridine into total phenol-extractable RNA is shown in Fig. 1A. The specific activity of the total RNA extracted from hormone-treated cells increased (after a lag of 30 min) markedly for 4 h, whereas control (vegetative) cell RNA reached a steady state 2–3 h after addition of the ^3H -uridine.

Figure 1B shows the effect of antheridiol on total protein accumulation. In the hormone-treated tissue a lag of 2–3 h was observed before the rate of incorporation of ^3H -leucine increased considerably. Conversely, incorporation of ^3H -leucine into protein in vegetative mycelia showed no detectable lag.

Total phenol-extractable RNA, from either the whole cell homogenate, nuclei or preparations of polysomes was passed through an unmodified Sigma 38 cellulose column²³, and the poly(A)-rich fraction was eluted with deionised H_2O (Fig. 2). The fraction eluted with H_2O (cellulose-bound) was found to be rich in adenylic acid (Table 1). The cellulose-bound fraction from both the hormone-treated and vegetative mycelia had greater than 33.5% AMP residues (Table 1). This was in contrast to the adenylic acid content found in the remainder of the cellular RNA. These values are similar to those reported for other eukaryotes^{8,17}.

The effect of antheridiol on the incorporation of ^3H -adenosine into poly(A)-rich RNA is shown in Fig. 1C. Antheridiol stimulated a three to four fold increase in the specific activity of the poly(A)-rich fraction after an initial lag period (2–3 h), whereas incorporation of label into the poly(A)-rich fraction of vegetative cells showed no lag, increased linearly, and reached a steady state after 3 h (Fig. 1C). The enhancement of incorporation between 3 and 4 h after addition of the hormone corresponds to the observation of the appearance of male sex organ initials. Addition of actinomycin D causes a rapid decline in the specific activity of the poly(A)-rich fraction within 1 h (Fig. 1C), suggesting

that total poly(A)-rich RNA accumulation is affected by actinomycin D and that the life span of a given poly(A)-rich molecule is very short (less than 1 h). Cordycepin, a drug which is known to affect the post-transcriptional addition of poly(A)⁹, rapidly inhibited the incorporation of ^3H -adenosine into *Achlya* poly(A)-rich RNA (Fig. 1C). This suggests that the mechanism for the addition of the poly(A) fraction to *Achlya* presumptive mRNA is similar to the mechanism operative in mammalian cells.

In order partially to insure that our incorporation studies were in fact measuring mRNA accumulation and not rapid changes in nucleotide pool sizes, we compared incorporation of label into poly(A)-rich RNA with the effect of antheridiol on leucine incorporation (Fig. 1B and 1C). Again, an enhancement of the total hot trichloroacetic acid (TCA) precipitable counts was observed when compared with the control. The rapid increase in specific activity of the total protein between 3 and 4 h corresponded to the rapid increase observed in total incorporation into poly(A)-rich RNA. These data indicate that antheridiol stimulates the accumulation and possibly the synthesis of an adenylic-rich fraction of cellular RNA. This enhancement of the total specific activity of the RNA fraction was observed whether the RNA was isolated from the total cell homogenate, from purified nuclei or from polyribosomes.

During male sex organ differentiation in *A. ambisexualis* (E87), the external morphogenetic stimulus, antheridiol, activates the accumulation of a class of RNA, presumably messenger, which is required for the initiation of the antheridial branches. The addition of actinomycin D and of cordycepin inhibits the accumulation of the messenger-like fraction and also prevents normal morphological differentiation. Similar stimulation of mRNA synthesis by oestrogen and progesterone has been reported during chick oviduct development²⁴.

If the poly(A)-rich RNA of *Achlya* is in fact mRNA, then it would be expected that differing size transcripts would be synthesised for differing size gene products. In an attempt to determine the relative size of the poly(A)-rich RNA from *Achlya*, polyacrylamide electrophoresis of the

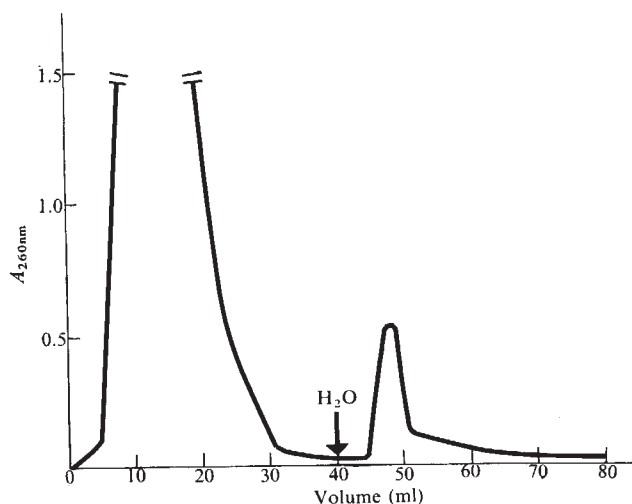


Fig. 2 Chromatography of *A. ambisexualis* RNA on Sigma 38 cellulose. RNA was extracted in SSC, 1% SLS precipitated in the presence of 0.3 M LiCl and taken up in deionised H_2O (1 ml). RNA concentration and radioactivity determined as in Fig. 1. The RNA was diluted to 10 ml with 10 mM Tris-HCl (pH 7.6), 500 mM KCl, 0.2 mM MgCl_2 , and 400 A_{260} units were applied to a 3 ml cellulose column²³. The column was washed with the above buffer until the A_{260} reading dropped below 0.02. Deionised water was then passed through the column to elute the poly(A)-rich RNA. This RNA was either used immediately or made up to SSC, 1% SLS and reprecipitated in ethanol.

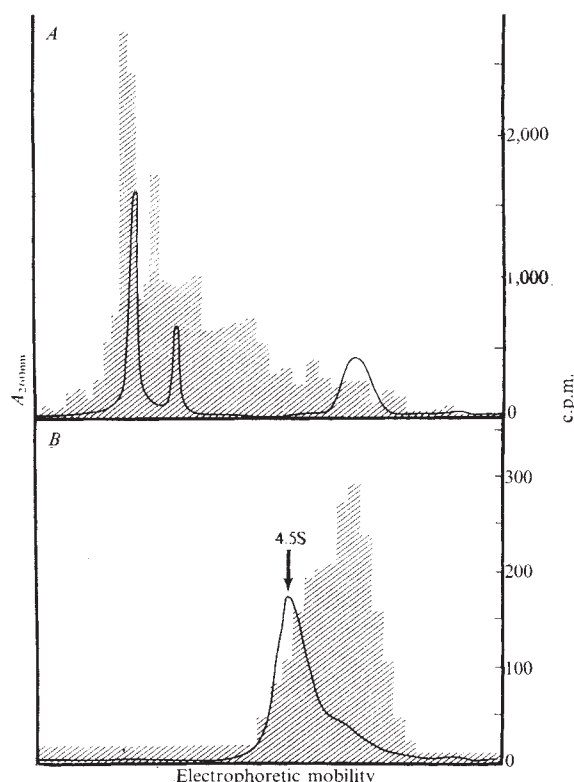


Fig. 3 Gel electrophoresis of *A. ambisexualis* poly(A)-rich RNA and RNAase digestion product. A, ^3H -adenosine-labelled poly(A)-rich RNA was isolated as described in Fig. 2 and reprecipitated with nonlabelled total *Achlya* RNA. Disc electrophoresis was performed using the procedure of Jaworski and Horgen²¹ in 2.4% gels. The gels were scanned with a Gilford linear transporter, frozen in dry ice and sliced with a Mickle gel slicer. The slices were solubilised with NCS (Nuclear Chicago) and counted in a Beckman LS-150 scintillation spectrometer. B, ^3H -adenosine-labelled poly(A)-rich RNA was digested for 30 min at 37°C in 10 mM Tris-HCl (pH 7.6), 50 mM KCl, 1 mM MgCl_2 with pancreatic RNase (Worthington 56.8 $\mu\text{g ml}^{-1}$). The hydrolysed solution was reprecipitated in ethanol with 100 μg *E. coli* tRNA (4.5S). The precipitated polynucleotides were washed once in ethanol and taken up in electrophoresis buffer²¹. The mixture of tRNA and *Achlya* poly(A) was layered onto a 5% gel and electrophoresed for 2 h at 6 MA per gel. Gels were scanned, sliced and counted as above. Similar results were obtained when cells were pulse labelled for 2 h with 15 $\mu\text{Ci ml}^{-1}$ ^{32}P . RNA was extracted from the 5 h hormone-treated cells or from vegetative control cells that had been labelled for 5 h. Both gave identical gel profiles.

Sigma 38 cellulose-bound fraction with *Achlya* 26S, 18S, 5S and 4S RNA present as markers was performed. Figure 3A indicates that the poly(A)-rich fraction ranged from approximately 30S to almost 4S. These results are similar to what has been reported in other eukaryotic cells^{16,17}.

To determine the size of the poly(A) tract we took advantage of the fact that polyadenylic acid is insensitive to hydrolysis by T_1 and pancreatic RNase A^{16,17}. After nuclease digestion and reprecipitation in ethanol, the *Achlya* poly(A) moiety was either subjected to alkaline hydrolysis or electrophoresis with *E. coli* 4.5S RNA as a marker.

In establishing if the RNase-resistant material of *Achlya* consisted of polyadenylic acid, it was subjected to base composition analysis after alkaline hydrolysis. The value for AMP was greater than 90%.

Coelectrophoresis with *E. coli* RNA showed that the poly(A) segment in *Achlya* migrated more rapidly than the 4.5S (25,000 daltons) marker suggesting a molecular weight of approximately 16–18,000 (Fig. 3B). This means that the poly(A) segment of *Achlya* presumptive mRNA is about 60 nucleotides long. This is significantly smaller than the poly(A)

tract in animal mRNAs^{9,15,16}, somewhat smaller than that found in cellular slime moulds¹², but very similar to that reported for yeast^{13,14}. It seems that evolution has resulted in progressively longer poly(A) pieces on the mRNA molecules in organisms with greater phylogenetic complexity.

The genus *Achlya* is a member of the most primitive group of eukaryotes that shows a developmental response to steroid hormones. We have demonstrated that in the male strain of *A. ambisexualis* (E87), associated with antheridiol stimulation of the development of sex organ initials, there is a corresponding effect of the hormone on the accumulation of a messenger-like fraction of RNA and on the accumulation of protein. These results suggest that the sterol affects morphogenesis by turning on the synthesis of mRNA which is then translated into protein required for the differentiation of the male sex organ initial.

We feel that *Achlya* provides a unique and valuable model system for the study of hormonal effects on development, both at the morphological and biochemical levels.

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Serological evidence of calicivirus transmission between marine and terrestrial mammals

THE recent isolation of two serotypes of San Miguel sea lion virus (SMSV), a calicivirus indistinguishable from vesicular exanthema of swine virus (VESV), from California sea lions (*Zalophus c. californianus*) and Northern fur seals (*Callorhinus ursinus*) points to: (1) the widespread distribution of SMSV, and (2) the role of marine mammals as possible reservoirs of viral diseases of terrestrial animals^{1,2}.

The latter hypothesis has now been assessed further by a serological survey for SMSV neutralising antibodies in sea lion and fur seal sera, collected in 1972, and in feral swine sera obtained in 1973. Individual blood specimens were collected from twenty mature female California sea lions on San Miguel Island, one of the Channel Islands off the coast of southern California. Further specimens were obtained from 120 immature (3-4-yr-old) bachelor bull fur seals from four hauling grounds, Reef, Tolstoi, Lukanin and Zapadni during the annual seal harvest on St Paul Island, a member of the Pribilof Group of Alaska. Blood samples were collected from eighteen feral swine during the annual hunt on Santa Cruz Island, another of the Channel Islands. After processing, serum specimens were stored at -20° C until tested.

Vero cell monolayers, used for either neutralisation tests or production of SMSV viruses, were grown in Eagle's minimum essential medium (MEM) supplemented with 5% foetal calf serum and antibiotics (penicillin 200 U ml⁻¹; streptomycin 100 µg ml⁻¹). Pools of the two serotypes designated SMSV-1MR and SMSV-2MR were produced by collecting inoculated Vero cell monolayers 18 h after infection and then titrating the supernatant fluids for 50% tissue infective doses (TCID₅₀ per 0.1 ml). The neutralising antibody test procedure was as follows. Serially diluted serum (inactivated by subjection to 56° C for 30 min) was mixed with equal volumes of virus suspension containing 100 TCID₅₀ per 0.1 ml, incubated at room temperature for 1 h, and 0.2 ml aliquots inoculated into each of four tubes of Vero cell monolayers per dilution tested. Inoculated tubes were then incubated stationary at 37° C and examined for cytopathic effect after 24, 48 and 72 h.

All twenty of the female sea lions had neutralising antibody titres to SMSV-2MR and eighteen had antibodies to SMSV-1MR (Table 1). The two distinct SMSV serotypes were isolated originally from different sea lions sampled on the same day. This together with serological evidence of SMSV antibodies against both isolates, confirms the simultaneous presence of two SMSV serotypes within the herd. This is not regarded as unusual for VESV; outbreaks have been reported where different VESV serotypes, attributed to mutation³, occurred in the same domestic swine herd within a short time.

In contrast to the findings in sea lions, no serological evidence of SMSV-1MR antibodies was found in northern fur seals on St Paul Island. This is puzzling because a virus serologically identical to SMSV-1MR was isolated from an emaciated pup from the Reef rookery on this island². One possible explanation is that the serum tested in our study was collected from 3-4-yr-old bachelor bulls who, due to their social behaviour, remained isolated from breeding adults and newborn pups. This would suggest that SMSV-1MR has been introduced recently into the fur seal herd on the Pribilof Islands.

One-third (40/120) of the fur seal sera tested had neutralising activity against SMSV-2MR (Table 1). Animals with antibody titres were from all four of the hauling grounds sampled. Although SMSV-2MR has not yet been isolated from any fur seals, serological evidence points to its past

TABLE 1 SMSV neutralising antibodies in sea lion, fur seal and feral swine sera

Antigen	Antibody titres	California sea lion (20)*	Northern fur seal (120)*	Santa Cruz feral swine (18)*
SMSV-1MR	<10	2	120	17
	10	7	0	1
	20	5	0	0
	40	5	0	0
	80	1	0	0
SMSV-2MR	<10	0	80	13
	10	1	20	0
	20	2	8	2
	40	5	10	1
	80	7	2	1
	160	4	0	1
	320	1	0	0

* No. of animals tested.

occurrence in the herd. It is important, however, that the same serotype of SMSV infected herds of two pinniped species as far apart as the Santa Barbara Channel and the Pribilof Islands. The annual fur seal migration from islands in the Bering Sea to waters off the coast of southern California and the presence of a small fur seal rookery on San Miguel Island, may account for the interspecies spread of SMSV.

Feral swine on Santa Cruz Island have also had experience with the same SMSV serotypes; five out of eighteen serum specimens tested had SMSV-2MR and one out of eighteen had SMSV-1MR neutralising antibodies. Wild swine have existed on the Channel Island for many years, where they could have made contact with pinnipeds by foraging on beaches or scavenging on carcasses. The presence of antibodies in swine sera indicates that viral transmission has occurred. The age of the swine sampled was unknown, but their weight showed that only heavier, presumably older, animals had neutralising antibodies.

The finding of SMSV antibodies in feral swine and marine mammals may throw light on the successive outbreaks of VES in 1932, 1933 and 1934 in domestic swine⁴. The isolation of SMSV and its endemicity in pinniped herds suggests that VES outbreaks in domestic swine herds originated from a marine source. This coupled with the observation that SMSV produces a vesicular disease characteristic of VES in swine⁵, and the finding of similar vesicles on fur seal flippers further substantiates inter-species transmission from a marine to a domestic animal^{5,6}. Thus an exotic disease of swine thought to have disappeared 17 yr ago is now believed to have been rediscovered in pinnipeds and certain feral swine.

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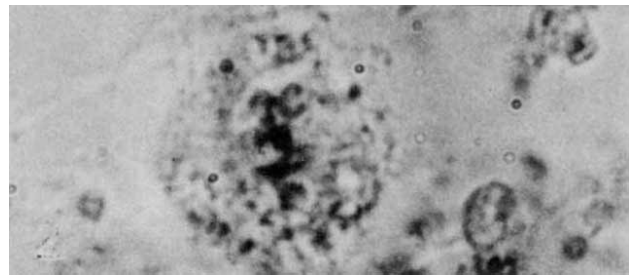


FIG. 2 Primary spermatocyte, metaphase, control: no stain is deposited in the spindle or cytoplasm ($\times 2,000$).

Distribution of adenosine triphosphatase during anaphase in cricket primary spermatocytes

THE idea that an adenosine triphosphatase (ATPase) may be involved in the generation of the force that moves chromosomes comes by analogy with other motile systems¹. In striated muscle, the ATPase is a part of heavy meromyosin². It forms cross bridges with actin filaments, and is an irreducible component in the mechanochemical process³. ATPase is also present in nonmuscular systems, such as slime moulds⁴,

myonemes⁵, cilia and flagella⁶⁻⁸, where it is essential for motility⁹⁻¹⁰.

An ATPase has been found in spindles isolated from sea urchins^{11,10-11}, but it is difficult to decide whether the enzyme has a functional role or is a contaminant, for the very low specific activity present in the spindle is the same as that in the cytoplasm¹¹. Other problems, such as the lability of the enzyme¹²⁻¹³, or the possibility that the spindle's force-producing mechanism is solubilised during isolation¹⁴ add to the difficulties. Furthermore, information necessary for the interpretation of spindle ATPase function cannot be detected in bulk spindle isolates. Such information would concern possible changes in the distribution of the same amount of enzyme within the spindle accompanying chromosome motion. A cytochemical approach to this problem, using spermatocytes of the domestic cricket *Acheta domesticus*, L., now indicates that the distribution of ATPase at anaphase differs from that at metaphase.

We cultured spermatocytes as described before¹⁵, except that gelatin was incorporated into the medium to make the cells adhere to the coverslip to prevent their loss during cytochemical procedures. (A detailed account of the use of gelatin will be given elsewhere.)

When the cultures were established, they were observed until the cells reached the desired stage of division. Then the coverslips bearing the cultures were immersed in a fixative containing: 1 part 2.5% glutaraldehyde and 0.002 M CaCl_2 in 0.03 M cacodylate buffer (pH 7.2); 9 parts 2.5% hydroxyadipaldehyde in 0.55 M sucrose buffered with 0.03 M cacodylate (pH 7.5). After 12 min coverslips were washed once in buffered sucrose (0.03 M cacodylate and 8.5% sucrose) at 0° C and pH 7.4 and then given two 10-min rinses in buffered sucrose at pH 4.0 (to remove soluble phosphate). They were stored overnight at 6° C in the buffered sucrose, pH 7.4.

The fixed spermatocytes were treated by a modified Padykula-Herman method¹⁷. Incubation in substrate medium was at 37° C for 1 h. To test substrate specificity, ATP was replaced by 1.4×10^{-3} M ADP, GTP or ITP.

After the cytochemical procedure cultures were mounted in glycerol and ringed with clear nail polish. Controls were parallel cultures incubated without substrate. Sulphydryl dependence was tested by incubation in cysteine-free medium containing 0.01 M *p*-hydroxymethyl benzoate (POHMB). Enzyme localisation was considered positive when a black stain was present after incubation in medium containing substrate but was not present in control medium (Fig. 2).

Figure 1 shows the ATPase distribution. In the metaphase primary spermatocyte (Fig. 1a) both the cytoplasm and the spindle area stained with the same intensity. The chromosomes and the Golgi, however, stained more heavily. When spermatocytes were in anaphase (Fig. 1b), the stain was not equally distributed throughout the spindle area—there was a clear area in the interzonal spindle region, between the separating dyads. The stain, however, remained

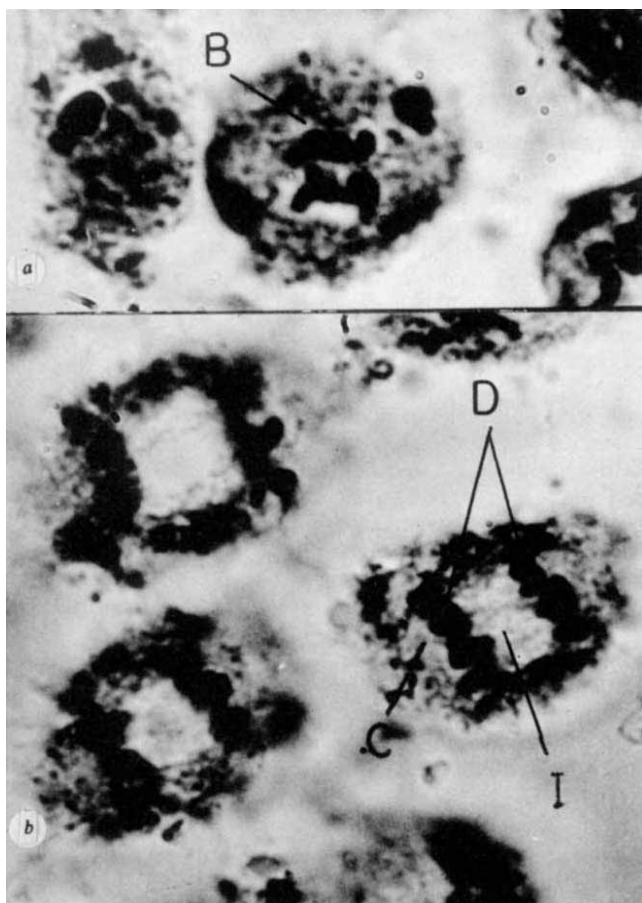


FIG. 1 The cytochemical distribution of ATPase in metaphase and anaphase primary spermatocytes from *Acheta domesticus*. ATPase is present in the stained areas ($\times 2,000$). a, Metaphase: the stain is equally distributed in the cytoplasm and spindle, but heavier deposits occur in the bivalents (B). b, Anaphase: stain is absent in the interzone (I) between the separating dyads (D) but present in the chromosomal fibre regions, (C).

in the chromosomal fibre region between the kinetochores and poles, and in the cytoplasm adjacent to the interzone. This difference in enzyme activity between the interzone and chromosomal fibre region persisted until the end of anaphase. The same pattern was found in secondary spermatocytes—equal distribution of enzyme activity between spindle and cytoplasm in metaphase and absence of activity in the interzone at anaphase. The stain remained undiminished in the chromosomes and other organelles. In control preparations, there were no differences between the interzones and half spindles under either bright field or phase contrast microscopy. With ADP (Fig. 3a), GTP (Fig. 3b) or ITP, both the interzone and the chromosomal fibre regions were stained in anaphase. In the presence of POHMB, the whole cell was more lightly stained and the degree of contrast between chromosomal fibre regions and interzones was reduced markedly (Fig. 4a and b). The Golgi, however, seemed to be unaffected by the presence of this sulphhydryl inhibitor.

These observations on metaphase spermatocytes are consistent with those from the sea urchin²¹, in that the ATPase was distributed equally between the spindle and the surrounding cytoplasm. They are not consistent with our failure to detect activity in spindles of root tip meristems¹⁷. This may reflect a difference in the two kinds of spindles (polar compared with apolar). But we used frozen sections of root tips, and have now determined that freezing denatures ATPase in spermatocytes. Thus our findings with root tips may have been artefacts.

The differences we observed in the anaphase distribution of ATPase were probably not a direct result of poor fixation of the interzone. Activity was evident in the interzone when nucleotide triphosphates other than ATP were used, and the interzone seemed to be morphologically intact when controls were viewed under phase contrast microscopy. We cannot, however, rule out the possibility that the ATP solubilises the interzone.

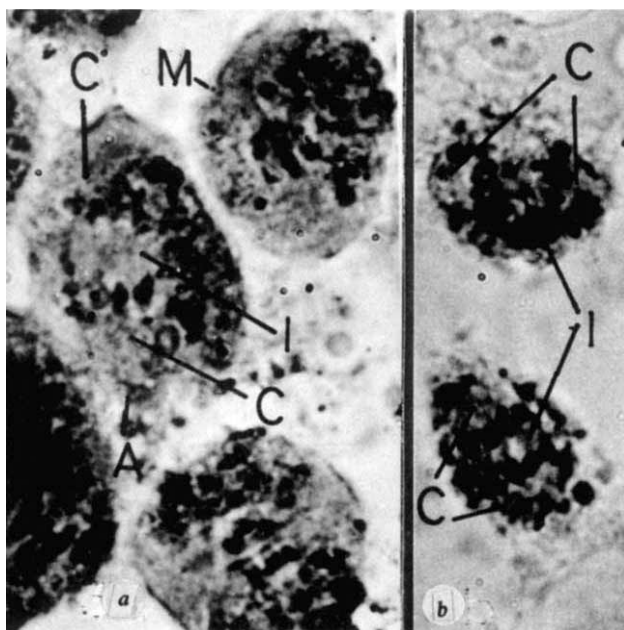


FIG. 3 The distribution of stain during anaphase when ADP and GTP replace ATP as substrate ($\times 2,000$). a, ADP: primary spermatocytes, metaphase and anaphase. The stain is deposited in the interzone (I) and in the chromosomal fibre (C) regions of the anaphase cell (A). In the metaphase cell (M) the stain is evenly distributed in the cytoplasm and spindle area. b, GTP: secondary spermatocytes. The stain is present in the interzone (I) and chromosomal fibre (C) regions during anaphase.

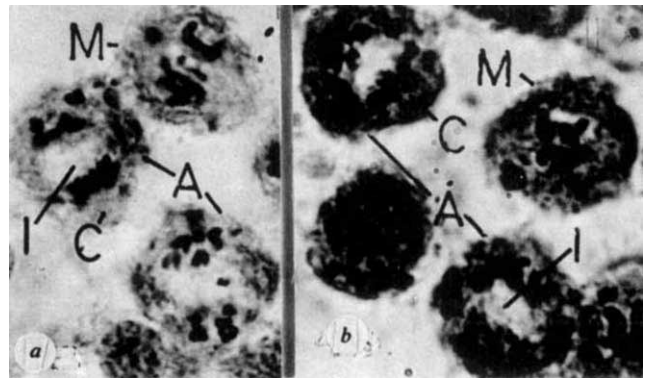


FIG. 4 Inhibition of ATPase by POHMB in metaphase and anaphase primary spermatocytes ($\times 1,000$). a, An ATPase determination in the presence of POHMB. Compare with (b). b, The control for (a). Cytochemical ATPase determined at the same time as (a) but without the inhibitor. The stain deposits are darker.

On the other hand, several facts suggest that the observed distribution of ATPase has functional significance. Thus, ATPase is present chiefly in the chromosomal fibre regions which are thought to generate the polarised forces to move the chromosomes or nonkinetochoric materials in anaphase^{18,19}. Furthermore, ATPase is absent from the interzonal spindle, where significant polarised forces are not detectable at anaphase^{18,19}, and its distribution in metaphase and anaphase follows the changes in birefringence believed to coincide with the production of spindle force²⁰.

The spermatocyte ATPase is sulphhydryl dependent, as is heavy meromyosin²¹. Actin-like filaments have been demonstrated in the spindles of grasshopper spermatocytes²², and in the spindles and cortex of crane fly spermatocytes^{23,24}. During anaphase these filaments are aligned parallel to the chromosomal fibres, but in the interzone they have a random orientation. Such orientation of actin filaments coincides with the reported distribution of ATPase and may explain the organisation of the ATPase in the chromosomal fibre region. If this were the case, then the interaction of actin and ATPase may be sufficient to generate forces that impart anaphase motion to chromosomes, and polarized motion to nonkinetochoric materials.

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Temperature immutability of adenylyl cyclase-coupled β adrenergic receptors

OBSERVATIONS from several laboratories have suggested that the well established classification of adrenergic receptors into α and β subtypes is not immutable. Several groups have reported that in isolated perfused frog hearts, stimulation of cardiac rate and contractility by catecholamines has the properties of a classical β adrenergic response when experiments are performed at warm temperatures (25°–37° C) but of an α adrenergic response when experiments are performed at cold temperatures (5°–15° C)^{1–3}. At warm temperatures, the order of potency of agonists in stimulating these preparations—isoproterenol > adrenaline > noradrenaline—is classical for a β adrenergic receptor. Similarly, effects of the catecholamines at warm temperatures are blocked by propranolol but not by the α adrenergic antagonist phentolamine. When the same experiments are performed at temperatures below 25° C, the order of potency of agonists is reversed to that characteristic of α adrenergic receptors. Also at lower temperatures α adrenergic antagonists such as phenoxybenzamine and phentolamine block the effects of adrenaline, whereas β adrenergic antagonists such as propranolol are ineffective. Gradations of response can be achieved by varying the temperature between 37° C and 10° C. Similar observations have been reported for the rat heart¹. Also in a dog heart–lung bypass preparation the β receptors seem to become ineffective at 15° C, whereas α receptors retain their effectiveness⁴. On the basis of such observations, Kunos *et al.*⁵ proposed that α and β adrenergic receptors may represent allosteric configurations of the same receptor macromolecule which could be modulated by among other factors, temperature.

During the past decade, many observations have supported the concept that the adenylyl cyclase system in various tissues provides an excellent biochemical model for study of the molecular properties of β adrenergic receptors⁶. In almost all situations where adenylyl cyclase is stimulated by catecholamines, the properties of this stimulation are those of β adrenergic receptors. Of course, virtually all experiments which have been reported have been performed at temperatures within the range of 30°–37° C. If, in fact, α and β adrenergic receptors merely represent temperature sensitive transitions in the state of a single macromolecule, then the ability of catecholamines to stimulate and of adrenergic antagonists to block stimulation of adenylyl cyclase should vary with temperature in a fashion analogous to that reported above. Accordingly, we have tested the ability of α and β adrenergic agonists and antagonists to stimulate and respectively block stimulation of adenylyl cyclase in several tissues from cold-adapted frogs, as well as from the hearts of several mammalian species, using a wide range of temperatures. Our findings do not support the contention that the adenylyl cyclase-coupled β adrenergic receptors are easily

convertible to α adrenergic receptors when the temperature varies.

Table 1 summarises results from several experiments performed with membranes prepared from dog and rat heart. In each case, stimulation of the adenylyl cyclase activity by catecholamines had the characteristics of a β adrenergic response at high (37° C) and low (15° C) temperatures. The β antagonist propranolol was an effective inhibitor of catecholamine-induced stimulation of the enzyme and the α adrenergic antagonist phentolamine was ineffective under all the conditions examined.

We next examined preparations from frog heart, since most of the evidence supporting the hypothesis of temperature conversion of β adrenergic receptors was obtained in this tissue. We had limited success however; using various conditions, we could demonstrate only marginal stimulation of the adenylyl cyclase activity by catecholamines in preparations of frog heart membranes.

But because the frog, which is cold blooded, seemed the most appropriate animal in which to test the possibility that the two forms of receptors are interconvertible, we have examined the properties of the adenylyl cyclase-coupled adrenergic receptors present in the frog erythrocyte. These are known to be typical β adrenergic receptors⁸.

As Figs 1 and 2 show, whether incubation was at 37° C or 10° C the stimulation of the frog erythrocyte cyclase was mediated by classical β adrenergic receptors. The order of potency of agonists was isoproterenol > adrenaline > noradrenaline > phenylephrine. Moreover, the stimulation of the enzyme by catecholamines could be blocked effectively by propranolol, a β adrenergic antagonist, whereas the α adrenergic antagonist phentolamine was virtually without effect. Similar findings were obtained with both summer and winter frogs, either warm or cold adapted.

TABLE 1 Effects of α and β adrenergic agonists and antagonists on adenylyl cyclase activity of canine and rat heart membranes at different temperatures

Additions Agonist Antagonist		Adenylyl cyclase activity (pmol cyclic AMP generated)			
		Dog heart membranes		Rat heart membranes	
		37°C	15°C	37°C	15°C
None	None	63.0	51.8	23.1	15.2
Isoproterenol	None	141.0	76.7	39.7	24.8
Isoproterenol	Propranolol	63.1	49.3	21.8	15.9
Isoproterenol	Phentolamine	131.7	84.3	40.4	21.8
Adrenaline	None	113.8	86.9	36.6	24.8
Adrenaline	Propranolol	66.6	43.9	17.4	14.9
Adrenaline	Phentolamine	123.6	88.2	35.2	24.9
Noradrenaline	None	102.3	82.2	35.7	23.8
Noradrenaline	Propranolol	51.6	39.8	21.2	15.2
Noradrenaline	Phentolamine	110.1	77.7	35.1	23.9
Phenylephrine	None	—	—	20.5	15.7
Phenylephrine	Propranolol	—	—	18.7	14.3
Phenylephrine	Phentolamine	—	—	24.6	19.5
None	Propranolol	56.5	39.1	22.1	18.4
None	Phentolamine	66.6	46.3	22.2	18.5

Ventricular myocardium (0.8 – 1.0 g) was homogenised in 20 ml of 0.25 M sucrose, 0.05 M Tris-HCl, pH 7.4 by ten strokes of a motor driven Potter–Elvehjem glass Teflon homogeniser. After filtration through cheese cloth, the homogenates were centrifuged in a Sorvall RC2B centrifuge for 10 min at 10,000g. Pellets were washed twice with the sucrose buffer and once with 75 mM Tris-HCl 25 mM MgCl₂, pH 7.4, before being assayed for adenylyl cyclase activity. Adenylyl cyclase was determined using a modification⁶ of Krishna's method⁷. Incubations were for 10 min at 37°C and for 30 min (canine heart membranes) or 60 min (rat heart membranes) at 15°C. Enzyme activity was linear for these periods of incubation at both temperatures. Reaction mixtures contained the following reagents in a total volume of 50 μ l: 25 mM Tris-HCl, pH 7.4; 10 mM MgCl₂; 1.5 mM ATP; 49–59 μ g of membrane protein; 0.1 mM cyclic AMP and an ATP regenerating system consisting of 5 mM phosphoenolpyruvate, 40 μ g ml⁻¹ pyruvate kinase and 20 μ g ml⁻¹ myokinase. Agonists and antagonists were added to a final concentration of 4×10^{-6} M. The values shown are the mean of duplicate determinations.

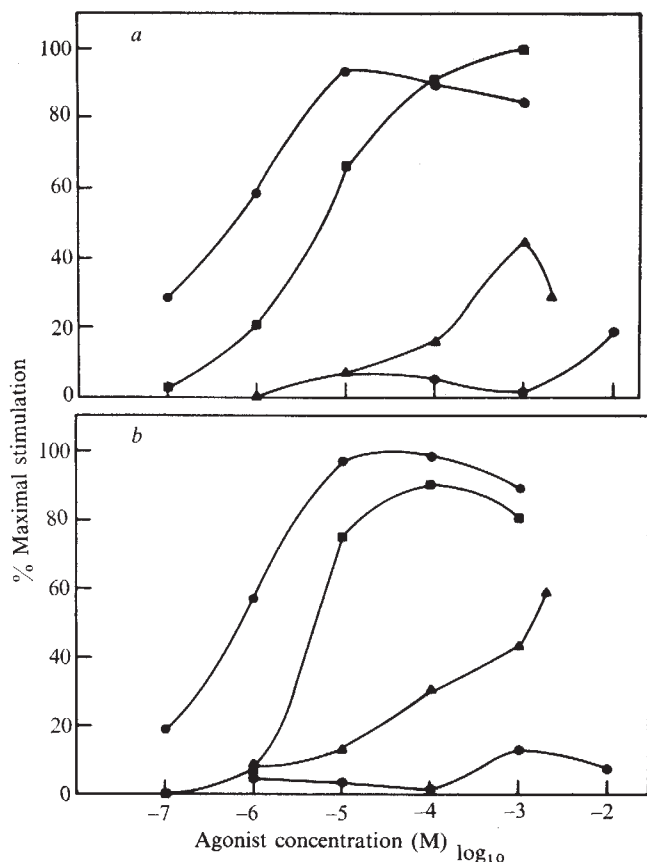


Fig. 1 Response of frog erythrocyte adenyl cyclase to adrenergic agonists at (a) 37°C and (b) 10°C. Isoproterenol (●), adrenaline (■), noradrenaline (▲) and phenylephrine (◐). Blood from cold-adapted southern jumbo grass frogs (Carolina Biological, Burlington, North Carolina) was collected and the red cells were washed three times with saline. Cells were lysed in 5 mM Tris-HCl, pH 8.1, buffer and the membranes were centrifuged at 18,000*g* for 15 min. The lysis process was repeated three times. Membranes were finally suspended in 75 mM Tris-HCl, 25 mM MgCl₂, pH 8.1 by homogenisation. Adenyl cyclase was assayed as described in Table 1, except that assays were carried out at pH 8.1 for 15 min at 37°C and for 60–90 min at 10°C. Cyclase activity was linear during these periods of incubation at both temperatures. Each sample contained 240–560 μ g of membrane protein. In these experiments the mean basal enzyme activity was 285 pmol per mg protein. Maximal stimulation refers to activity observed in the presence of a maximally effective concentration of catecholamine which was generally about five times the basal activity. Each value is the mean of four determinations.

These findings suggest that the adrenergic receptors coupled to adenyl cyclase in mammalian heart as well as in frog erythrocytes are typical β adrenergic receptors, the characteristics of which are not altered at a wide range of temperatures. These observations do not seem to support the previous physiological observations reported and the hypothesis that β adrenergic receptors are convertible to α adrenergic receptors when temperatures are lowered, especially in the tissues of the cold-adapted frog. It should be noted that we were unable to test specifically the validity of the hypothesis for the frog heart, the tissue for which the most data has previously been reported. We cannot explain why only a marginal stimulation of frog myocardial cyclase activity was observed with catecholamines. As noted above, however, comparable changes in the specificity of the adrenergic receptors with temperature variation have been reported for rat heart¹. In rat heart membranes, we saw no alteration in the adenyl cyclase-coupled β adrenergic receptors when the temperature was lowered.

Our observations raise objections to the hypothesis³ that α and β adrenergic receptors are, in general, easily convertible allosteric forms of the same macromolecule. Although our data do not specifically disprove the hypothesis for the limited case of the frog heart, they do negate the hypothesis for the adenyl cyclase-coupled β adrenergic receptors of another frog tissue, the erythrocytes, as well as the mammalian heart in several species. It should be noted that whereas previously reported studies involved intact tissue preparations, our results were obtained exclusively with sub-cellular membrane fractions. Thus, it is possible that the apparent discrepancies in the results are due to a requirement for an intact cellular structure to mediate the changes in receptor specificity. The mechanisms involved in such an effect, however, are not clear.

It has not been shown definitively that β adrenergic effects on the myocardium are mediated by adenyl cyclase and cyclic AMP⁹. Moreover, the function of the frog erythrocyte β adrenergic receptors is not known. Nonetheless, as demonstrated here, the receptors which mediate effects of catecholamines on adenyl cyclase in each of these tissues are clearly β adrenergic receptors and hence represent a valid biochemical model of β receptors for studies such as these.

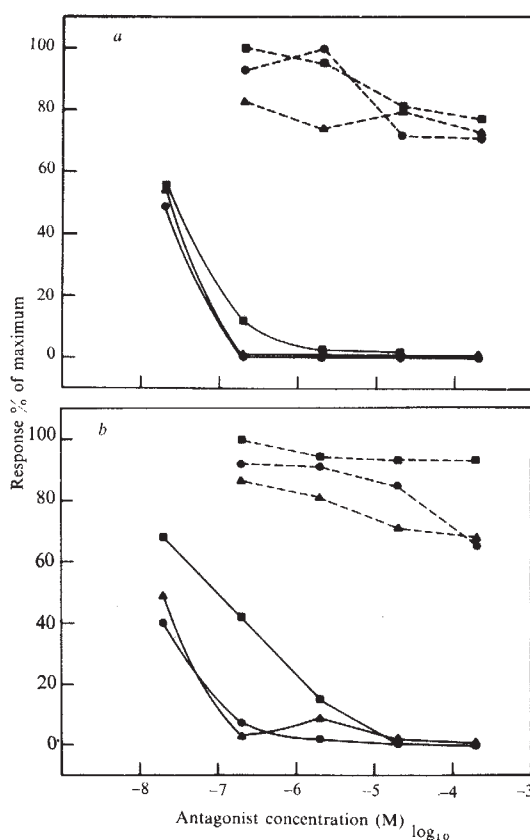


Fig. 2 Inhibition of catecholamine-induced activation of frog erythrocyte adenyl cyclase by adrenergic antagonists at (a) 37°C and (b) 10°C. Propranolol (—); phentolamine (---). The agonists were present at the following concentrations: isoproterenol, 1×10^{-6} M (●); adrenaline, 1×10^{-5} M (■) and noradrenaline $1-5 \times 10^{-5}$ M (▲). Methods of membrane preparation and adenyl cyclase assay are described in the legend to Fig. 1. Incubations were carried out at 37°C for 15 min and at 10°C for 90 min. Each assay contained 250–310 μ g of membrane protein. Maximum response refers to enzyme activity, in the presence of each agonist, at the stated concentration. 0% maximum response refers to activity in the absence of agonist, that is basal enzyme activity. In these experiments the mean basal activity was 207 pmol per mg protein and the concentration of agonists used gave usually a two to three-fold stimulation over basal activity. The results shown are the mean of four determinations.

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FIG 2 A bacteroid in the ooplasm of an actively vitellogenic oocyte from the active ovary of a female 4 d after emergence. Note the fibrils and ribosomes in the bacteroid. ($\times 54,500$.)

Bacteroids in the ovaries of a tsetse fly

SYMBIONTS of *Glossina* previously described by Wigglesworth¹ as Gram-negative intracellular bacteroids are located in a group of specialised cells, the mycetome, in the midgut. Their intracellular nature is well known from studies with the light¹⁻³ and electron⁴ microscopes (see Fig. 4). Roubaud⁵ demonstrated bacteroids in the mycetome of the larval midgut of *G. palpalis*. He was unable to determine the mode of transmission, but suspected the involvement of the milk gland, as have other authors^{5,6}. Recent work on the ultrastructure of the milk gland has demonstrated that bacteroids occur extracellularly in the lumen of the gland⁷, thus adding

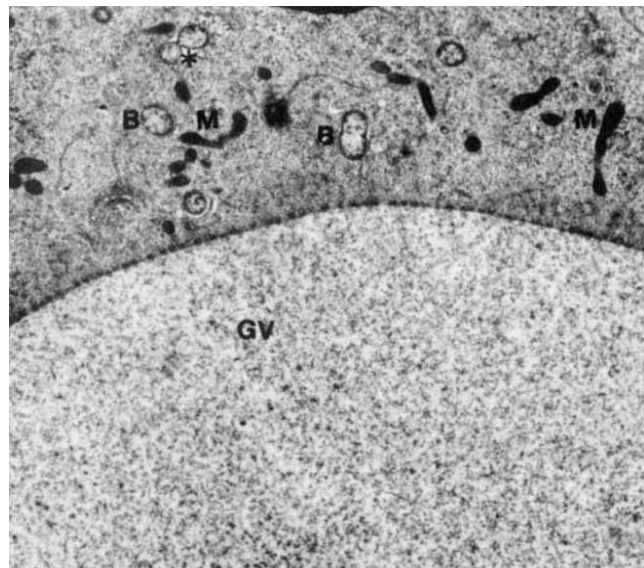


FIG. 1 An electron micrograph of part of a young oocyte from the inactive ovary of a newly emerged fly. Note the numerous bacteroids (B) in the ooplasm, which are clearly distinguishable from the mitochondria (M). A dividing bacteroid (*) is also visible. GV, germinal vesicle. ($\times 7,000$.)

support to the notion that transmission from one generation to the next may occur through the secretions of the milk gland. Bacteroids have not, however, been located in the cells of the milk gland, and it remains uncertain whether this route of transmission is used.

We report here for the first time the presence of intracellular bacteroids in the ovary of *G. austeni*, and suggest that their presence indicates the transovarian transmission of symbionts found in the larval and adult mycetome.

The ovaries of mated *G. austeni*, reared by the procedure of Tobe and Davey⁸ were fixed⁹ on the day of emergence and every second day thereafter up to 28 d after emergence, thereby spanning two pregnancy cycles. Both ovaries of two or three flies were fixed at each time, and tissues were embedded in Epon-Araldite¹⁰. Sections were cut on an LKB ultratome using glass knives, collected on uncoated copper grids, stained with uranyl acetate and lead citrate and examined in a Zeiss EM9S electron microscope.

Bacteroids were present in the cytoplasm of oocytes (Figs 1 and 2) and nurse cells (Fig. 3) of the ovary throughout the reproductive cycle. Similar bacteroids were seen occasionally in follicle cells and sheath cells of the ovary. Their surface structure and internal morphology resemble Gram-negative bacteria¹¹. They are oblong, approximately 2 μ m long, and contain a fibrillar network and ribosomes. They are bounded by a plasmalemma, an intermediate layer, and an outer double membrane. In addition, a host cellular membrane surrounds the whole structure (Figs 2 and 3). Dividing bacteroids were sometimes observed (Fig. 1). The presence of bacteroids in ovaries from newly emerged flies from pupae received from the Tsetse Research Laboratory at Langford in Britain ruled out the possibility of the microorganisms originating as an infection due to the culture conditions in our laboratory. The bacteroids do not elicit degenerative responses from the host tissues: they were not found to be associated with lysosomes or autophagic vacuoles. Since the viability of the subsequent offspring from our flies was not affected, we can only conclude that these are normally occurring symbionts with some functional significance. Previous studies on oogenesis in tsetse flies¹²⁻¹⁴ have revealed no symbionts, but the methods used gave insufficient resolution.



FIG. 3 Bacteroids in the cytoplasm of a nurse cell from a newly emerged female. Note the presence of host membrane (arrow) around the bacteroid. ($\times 39,500$.)

While ovarian symbionts may be important to the processes of oogenesis or fertilisation¹⁵, it is perhaps more likely that these organisms constitute a source for the symbionts of the larval and adult midgut. Certainly such a function is well known in other arthropods⁶. The symbionts present in the nurse cells may be transported through intracellular bridges to the oocyte, where they would be conveniently placed for localisation in the presumptive midgut cells of the embryo. The symbionts of the ovary are similar in structure to those in the midgut mycetome of the adult (Fig. 4), but are considerably smaller. Moreover, the midgut forms appear not to be enclosed in a host membrane⁴. However, the symbionts of the ovary resemble those of the midgut more

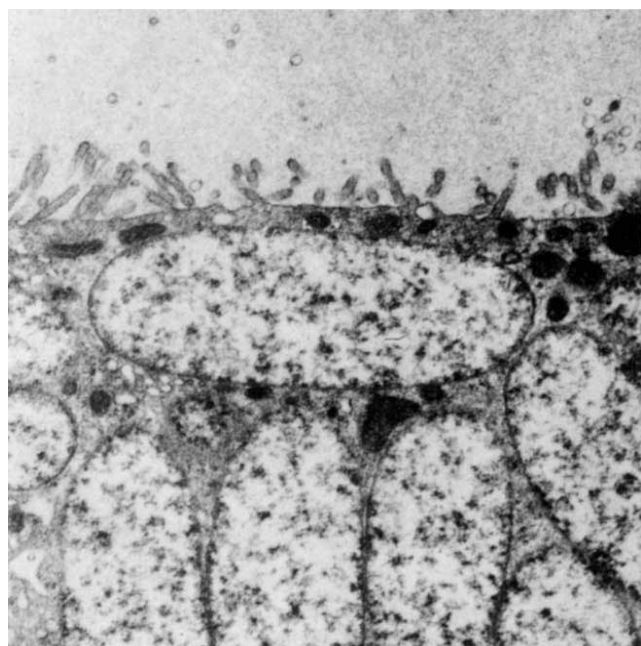


FIG. 4 Bacteroids in the apical cytoplasm of the mycetome of an adult female. ($\times 13,000$.)

closely than do the microorganisms reported in the lumen of the milk gland⁷.

Although we do not discount the possible role of milk in the transmission of symbionts to the larva, our findings provide concrete support for transovarian transmission as an alternative or parallel mechanism. This means of transmission from one generation to the next does not require the externalisation of the bacteroids into the lumen of the milk gland, and their subsequent penetration into the cells of the gut of the larva. While the presence of symbionts in the ovaries of the adult, and hence the possibility of transovarian transmission, is clear, a decision as to whether the symbionts are the progenitors of those found in the mycetome of the larval and adult midgut awaits a detailed ultrastructural study of their fate during embryonic development.

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Uptake of horseradish peroxidase by frog photoreceptor synapses in the dark and the light

INDIRECT evidence suggests that vertebrate photoreceptors decrease their release of a depolarising transmitter substance in response to the absorption of light. Vertebrate photoreceptors¹⁻³ and most horizontal cells^{2,4} hyperpolarise in response to light and show an increased membrane resistance. In addition, transretinal currents that depolarise receptor terminals also depolarise horizontal cells⁵⁻⁷. Finally, magnesium, known to decrease transmitter release in neuromuscular⁸ and other synapses⁹, hyperpolarises skate¹⁰ and turtle¹¹ horizontal cells. We now report more direct evidence that the photoreceptors are synaptically active in the dark and that this activity can be diminished by light.

Active synapses can be labelled with macromolecular tracers. This has been established using horseradish peroxidase to study lobster neuromuscular junctions¹²; by work with vertebrate neuromuscular junctions studied with peroxidase^{13,14} and dextrans¹⁵; by investigations of cultured central

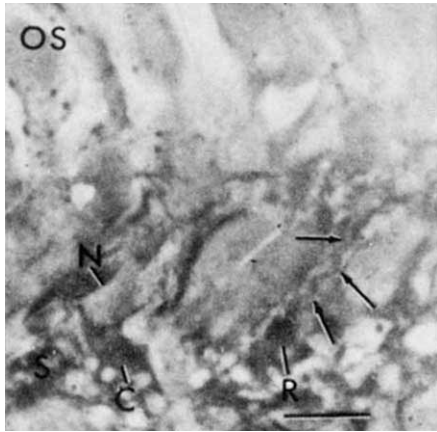


Fig. 1 Phase contrast photomicrograph of a thick section of a retina exposed to peroxidase for 60 min in the dark. At R, a rod cell synapse is seen, made dark by its content of peroxidase reaction product; note the thin characteristic stalk (arrows) by which the synapse is attached to the nuclear region (this latter region is not seen at this plane of focus). C indicates a cone cell synapse, also containing reaction product; as expected for this cell type, the synapse is quite close to the nuclear region (N). OS indicates the level of the outer segments. S indicates the level of the synaptic layer. The bar represents 10 μ m.

nervous tissue⁹, and by studies of neurosecretory systems¹⁶. The essential findings in these studies and some related work (for review see ref. 9), were that synaptic terminals could be shown to take up tracers into small synaptic vesicles and other membranous structures when stimulated to release transmitters by electrical or chemical means. Uptake into synapses that were not active in transmitter release was slight.

For the present work, isolated frog retinæ were prepared by standard procedures and maintained under various conditions of illumination in either Ringer or Na-aspartate Ringer solution¹⁷. Aspartate depolarises the postsynaptic membranes and thus isolates receptor potentials¹⁸⁻²⁰. The retinæ were placed in a chamber of an extracellular recording system so that we could monitor the electrical activities of the receptors. Horseradish peroxidase (Sigma Type II) at a concentration of 0.5% was included in the bathing medium. After suitable periods of exposure, the retinæ were rinsed briefly in Ringer, fixed in 2.5% glutaraldehyde in cacodylate buffer, cut in small pieces, incubated to demonstrate peroxidase activity²¹, postfixed in osmium tetroxide, embedded in Epon and prepared for light and electron microscopy. Cytochemical controls, such as preparations incubated in a peroxide-free cytochemical medium or retinæ never exposed to exogenous tracers but incubated in full medium, showed no reaction product.

The synapses of frog photoreceptors appear to be active in the dark. When retinæ were maintained in the dark for 30-90 min, extensive uptake of peroxidase by the synapses was observed. This was readily detectable by light microscopy of thick sections cut from the Epon blocks (Fig. 1) where reaction product is seen as dark brown diffuse or granular deposits within the synapses. It was often possible to determine whether a particular synapse belonged to a rod or a cone receptor by focusing up and down carefully, and thus following the cell from the synapse to its outer segment. Furthermore, the position of the nucleus of the cell making the synapse also helped show whether it was a cone or rod synapse. Most rod nuclei are located in the distal layer of the photoreceptor nuclear region while cone nuclei are found in the proximal layer²². (Most of the green rod nuclei are also located among the cone nuclei. These cells, however, comprise only about 8% of the receptor population

while cones make up about 42% and red rods 50% (ref. 23). In the electron microscope, most, if not all, the synapses from dark retinæ were seen to have taken up peroxidase into numerous vesicles (Fig. 2).

When retinæ were maintained in the light (for 30-90 min) at an intensity that decreased rod sensitivity substantially relative to cone sensitivity, markedly fewer synapses were observed to have taken up extensive amounts of tracer. It was clear from careful light microscopy that many of the active synapses were of cone cells and many of the inactive ones were of rod cells. (It was impossible to designate all synapses as rod or cone because of such factors as inappropriate plane of section. We are confident, however, that few rods had appreciably active synapses under these conditions.) In the electron microscope (Fig. 3), many synapses were observed to have contents of peroxidase comparable with that found in dark synapses (synapse A) while many others (synapse B), took up little tracer. Synapse A is quite close to its nucleus, indicating that this is probably a cone.

Another procedure used is known to decrease the frog's rod sensitivity relative to that of the cones, but it does not require the continuous presence of light. Retinæ were exposed for 5 min to very high intensities of light that bleach about 90% of the visual pigment²⁴. The preparations were allowed to recover for 30 min in the dark and then for the first time they were exposed to peroxidase (for 30-45 min in the dark). Under these conditions, the rods of an isolated retina permanently lose sensitivity because they cannot regenerate their visual pigment in the absence of the pigment epithelium²⁵⁻²⁸. Cones, on the other hand, can regenerate their pigment²⁹ and recover nearly all their sensitivity¹⁷. The results we obtained were similar to those found in the continuous light condition. In the light microscope, many of the active synapses seemed to belong to cone cells and many of the inactive ones were from rod cells. In the electron microscope, the preparations were essentially similar to the continuous light preparations (compare Fig. 3).

The previous work on tracer uptake by neuronal synapses^{9,12-16} strongly suggests that such uptake reflects the membrane rearrangements attendant on transmitter release. As always, the presence of a tracer molecule could conceivably

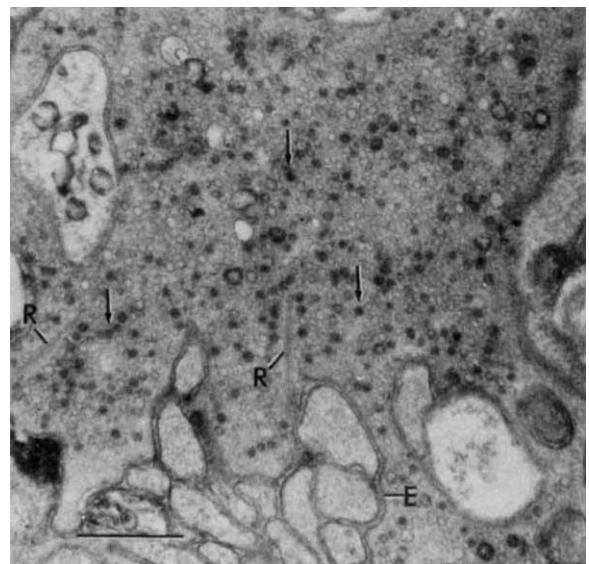


Fig. 2 Synapse from a retina exposed to peroxidase in the dark for 90 min. Reaction product is present in about 35% of the small vesicles. A few labelled vesicles are indicated by arrows; it may eventually prove of interest that some of these vesicles are clustered near the synaptic ribbons (R). Reaction product in extracellular space is seen at E. The bar represents 0.5 μ m.

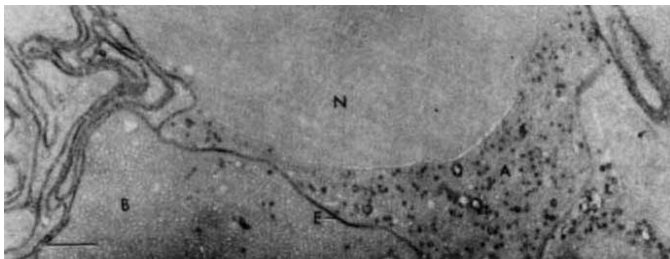


FIG. 3 Synapses from a retina exposed to peroxidase in the light for 45 min. Synapse B shows reaction product at its surface (E) but only 3% of the vesicles are labelled. In synapse A, 30% of the vesicles show reaction product. Note the edge of the nucleus (N) abutting on the synaptic region of A. The bar represents 0.5 μ m.

alter the normal behaviour of the cells. However, our electrophysiological recordings indicate that the presence of peroxidase had little or no effect on the receptor responses. In addition, since the rods do not take up peroxidase in the light and bleach conditions, uptake cannot be explained as simply an artefact of the presence of the tracer.

Thus, our findings indicate that, at least in the isolated retinae, the photoreceptors are synaptically active in the dark. On exposure to lights (continuous or brief high intensity) that have been shown to desensitise frog rod receptors^{24,30}, rod synapses become quiescent but cone synapses remain active. If we make the reasonable assumption that synaptic activity is controlled by membrane potential, then our results are consistent with the evidence indicating that rod receptors are relatively depolarised in the dark and hyperpolarised during continuous light exposure^{31,33}. However, more than 30 min after light exposures that bleach substantial rod pigment, we found little or no rod synaptic activity while physiological experiments on skate³⁴ and fish³⁵ retinae can be interpreted as indicating that synaptic activity returns fairly quickly to dark levels. Further work on potential changes in the frog retina is needed to determine the time course of changes under the conditions we have used.

Preliminary reports of these findings have been presented already^{36,37}. We thank Fe Reyes for technical assistance. This work was supported by a grant from the National Institute of Neurological Disease and Stroke and by a US Public Health Service training grant.

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Human lymphocyte-dependent antibody mediated cytotoxicity and direct lymphocyte cytotoxicity against non-HL-A antigens

EXPRESSION of the major transplantation antigens in humans is generally considered to be controlled by the genes of the *HL-A* region¹⁻⁴. Skin, kidney and bone marrow transplants usually survive longer when donors and recipients are HL-A identical than when they are not⁵⁻⁷. However, the fact that grafts are still rejected with HL-A identity and negative mixed lymphocyte reactivity (MLR)⁸⁻¹¹ strongly suggests the presence of minor transplantation antigens. The major problem to date has been the development of *in vitro* assay systems to detect these antigens.

Attempts to identify presensitisation of potential graft recipients to non-HL-A antigens have generally failed when they have involved testing for complement-dependent antibody to donor cells^{12,13}. On the other hand, there are reports of cytotoxicity, mediated by sensitised lymphocytes from multiply transfused patients, against non-HL-A antigens^{13,14}. We have now found that patients who have received multiple transfusions develop lymphocyte-dependent antibody (LDA) as well as cytotoxic lymphocytes against non-HL-A antigens. These responses occur in spite of negative MLR.

Three patients, two males and a female, were studied: two suffered from aplastic anaemia and one from a pre-leukaemic state with pancytopenia. They all received multiple blood and platelet transfusions from unmatched

TABLE 1 Family HL-A genotypes

Family No.	Father	Mother	Patient	Siblings†		
1	1, 8/2, 8	(10, W10/3, W10)*	2, 8/10, W10	2, 8/10, W10;	1, 8/10, W10;	2, 8/3, W10
2	3, 7/23, W5	(2, W22/?3W17)	3, 7/2, W22	3, 7/2, W22;	3, 7/2, W22;	?3, W5/?3, W17
3	3, W5/10W18	(10, 12/9, 22)	3, W5/10, 12	3, W5/10, 12;	3, W5/9, 22	

* Parentheses indicate a deduced genotype in a parent who was not typed.

† HL-A typing was performed for families 1 and 2 by Dr P. Terasaki and the NCI Human Histocompatibility Typing Center maintained at Microbiological Associates, Inc. with identical results. Family 3 was typed only by Dr P. Terasaki

TABLE 2 Mixed lymphocyte culture stimulation indices for HL-A identical siblings*

Responder	Stimulator	Ratio†
Patient 1	Sibling	1.1
	Unrelated	5.8
Patient 2	Sibling	1.0
	Unrelated	28.8
Patient 3	Sibling	1.3
	Unrelated	7.6

* MLR was performed as previously reported¹⁶. There were 2×10^5 responding lymphocytes per tube. Stimulating cells ($2 - 10 \times 10^5$ per tube) were treated with mitomycin C.

† Results are expressed as the ratio of d.p.m. in cultures stimulated with allogenic cells to d.p.m. of cultures stimulated with autologous cells, that is $A + Bm/A + Am$. A ratio ≥ 2 represents stimulation. Incubation time was 4-7 d and the ratio was determined from the time at which the peak stimulation was achieved with that pair of individuals.

donors and from their HL-A identical sibling. HL-A typing data (Table 1) demonstrated genotypic identity between each patient and sibling.

Cytotoxicity mediated by sensitized lymphocytes and by LDA was measured by rapid release of ^{51}Cr as described before^{13,15}. Target and attacking cells were obtained by passing heparinised blood over nylon columns and by incubating nonadherent cells with Plasmagel. Target cells were treated with NH_4Cl -Tris buffer to eliminate red blood cells and then were labelled with ^{51}Cr (200-400 μCi given to 10^7 cells for 30-60 min). To test for direct cellular cytotoxicity, 5×10^4 ^{51}Cr -labelled target cells were incubated with 3×10^6 attacking lymphocytes (1 ml) in 35mm plastic dishes for 4 h on a rocking platform in a CO_2 incubator. To test for LDA-mediated cytotoxicity, ^{51}Cr -labelled target cells were incubated with serum containing LDA for 30 min, and then washed by centrifugation. Target cells (10^4) were placed in dishes with 10^6 normal attacking lymphocytes (1 ml) (test group) or media alone (control group). After 4 h the contents of the dishes were transferred to tubes; 1 ml of cold balanced salt solution + 10% foetal calf serum was added and tubes were centrifuged (800g, 10 min). Aliquots were taken for counting of ^{51}Cr release. Immune percentage lysis was calculated by comparing the ^{51}Cr released from target cells during the 4 h incubation with the maximum amount released by freezing and thawing target cells. The following equation was used to calculate immune percentage lysis:

$$\frac{\text{Average test } ^{51}\text{Cr release} - \text{Average control } ^{51}\text{Cr release}}{\text{Freeze-thaw } ^{51}\text{Cr released} - \text{machine background}} \times 100$$

Standard error:

$$\frac{1}{\text{freeze-thaw } ^{51}\text{Cr release}} \left[\left[\frac{\text{variance}}{\text{sample No.}} \right]_{\text{test group}} + \left[\frac{\text{variance}}{\text{sample No.}} \right]_{\text{control}} \right]^{1/2}$$

Lymphocytes from each patient failed to be stimulated in MLR by cells from his other HL-A identical sibling (Table 2). MLR tests were technically satisfactory as demon-

strated by stimulation of these patients' lymphocytes by cells from a third party. On the other hand, serum from each of these patients supported LDA-mediated cytotoxicity against target cells from his HL-A identical sibling and against third party target cells from a donor not matched with the patient for HL-A antigens (Table 3). LDA-mediated cytotoxicity against non-HL-A antigens was directly proportional to the relative antibody concentration, and the activity was stronger against unrelated targets. All three sera were negative when tested against autologous freshly explanted lymphocytes (Table 3). These target cells were lysible in LDA reaction as demonstrated with LDA-containing serum from unrelated multiply transfused donors.

The three pairs of donors were tested for direct lymphocyte cytotoxicity, and in each case the patient's lymphocytes lysed ^{51}Cr -labelled target cells from the HL-A matched sibling and from an unrelated donor (Table 4). These attacking lymphocytes were not cytotoxic for autologous targets, thus demonstrating specificity.

We have demonstrated that three patients who were supported by blood and platelet transfusions from their HL-A identical siblings developed LDA against non-HL-A

TABLE 3 LDA-mediated cytotoxicity against targets from HL-A identical siblings

Family	Source of target lymphocytes*	Source of coating serum†	Source of normal attacking lymphocytes‡	Target cell immune damage % lysis $\pm$ s.e.§
1	Identical sibling	Media	Father-1	-0.6 $\pm$ 1.7
	Identical sibling	Patient-1	Father-1	10.1 $\pm$ 1.1
	Father-1	Media	Father-1	1.9 $\pm$ 3.4
	Father-1	Patient-1	Father-1	17.6 $\pm$ 3.6
	Patient-1	Media	Patient-1	-0.6 $\pm$ 1.2
	Patient-1	Patient-1	Patient-1	0.8 $\pm$ 1.6
2	Identical sibling	Media	Unrelated donor	1.4 $\pm$ 1.1
	Identical sibling	Patient-2	Unrelated donor	20.2 $\pm$ 0.9
	Patient-2	Media	Unrelated donor	1.3 $\pm$ 1.0
	Patient-2	Patient-2	Unrelated donor	-0.6 $\pm$ 1.0
	Patient-2	Patient-1	Unrelated donor	15.6 $\pm$ 0.7
	Identical sibling-3	Media	Unrelated donor	1.1 $\pm$ 1.7
3	Identical sibling-3	Patient-3	Unrelated donor	16.1 $\pm$ 1.7
	Patient-3	Media	Unrelated donor	1.0 $\pm$ 0.8
	Patient-3	Patient-3	Unrelated donor	1.6 $\pm$ 0.3
	Patient-3	Patient-2	Unrelated donor	50.0 $\pm$ 0.9

* The concentration of target cells was 10^4 per dish; all numbers identify a specific family.

† Serum dilutions were: family 1, 1:20; families 2 and 3, 1:2.

‡ There were 10^6 attacking lymphocytes per dish.

§ Damage after a 4-h assay. Values were corrected for ^{51}Cr releases from target cells incubated in media alone. ^{51}Cr releases from antiserum-treated target cells varied between 7% and 30%; ^{51}Cr releases from untreated cells varied between 6% and 13%.

TABLE 4 Direct cellular cytotoxicity against targets from HL-A identical siblings

Experiment	Source of target lymphocytes*	Source of attacking lymphocytes†	Target cell damage % lysis $\pm$ s.e.‡
1	Identical sibling-1a	Patient-1	22.3 $\pm$ 1.1
	Identical sibling-1a	Identical sibling-1b	-0.4 $\pm$ 0.8
	Identical sibling-1b	Patient-1	22.6 $\pm$ 1.9
	Identical sibling-1b	Identical sibling-1a	-1.9 $\pm$ 1.5
	Father	Patient-1	29.1 $\pm$ 2.3
	Father	Father	-5.9 $\pm$ 2.3
	Patient-1	Patient-1	-0.8 $\pm$ 0.4
	Identical sibling-2	Patient-2	8.04 $\pm$ 0.7
2	Identical sibling-2	Identical sibling-2	1.9 $\pm$ 0.6
	Non-identical sibling-2	Patient-2	13.2 $\pm$ 1.0
	Non-identical sibling-2	Non-identical sibling-2	-0.5 $\pm$ 0.9
	Patient-2	Patient-2	0.03 $\pm$ 0.8
3	Identical sibling-3	Patient-3	6.4 $\pm$ 0.4
	Identical sibling-3	Non-related donor	-1.9 $\pm$ 0.7

* There were 5×10^4 target cells per dish. Numbers refer to specific families. Siblings 1a and 1b were HL-A identical with the patient. Results from family 2 were published in ref. 13.

† There were 3×10^6 attacking cells per dish. Numbers identify specific families.

‡ Damage after a 4-h assay. Values were corrected for 'spontaneous' ^{51}Cr release from target cells incubated in media alone. These spontaneous ^{51}Cr releases varied from 5.6% to 25.1% lysis. Media (Eagle's essential) contained 10% heat-inactivated foetal calf serum.

antigens on their sibling's target cells. In addition, these patients developed cytotoxic lymphocytes against cells from the HL-A matched siblings. Together with two other cases we described before¹³, we have now five patients who demonstrated direct cellular cytotoxicity against non-HL-A antigens. LDA and cytotoxic lymphocytes did not mediate damage to autologous target cells, demonstrating the specificity of the assays.

It was not surprising that lymphocytes from each patient were not stimulated in MLR when incubated with his or her sibling's cells, while patients' lymphocytes were adequately stimulated by third party cells; cell donors presumably shared the same HL-A-linked MLR genes. This suggests that HL-A-linked MLR gene differences are not essential for development of cellular and humoral immune responses to transplantation antigens. In support of this concepts are results reported with humans^{17,18}, and dogs¹⁹, involving transplants between siblings genotypically matched for major histocompatibility antigens and MLR negative. In the one human pair, a skin graft was rejected by the recipient¹⁷. In a separate human study, severe graft versus host (GvH) disease developed in the recipient of a bone marrow graft¹⁸. Likewise, six of sixteen dogs developed fatal GvH disease following marrow grafting¹⁹. The recipient of the skin graft and the donors of bone marrow in all these cases were not preimmunized by blood transfusions. The possibility that MLR differences are not essential for cellular immune responses does not agree with reports that HL-A-linked MLR genetic differences are necessary for primary *in vitro* sensitisation of human peripheral blood lymphocytes against allogeneic transplantation antigens^{20,21}. The studies assessed sensitisation by both MLR and cell-mediated cytotoxicity tests. Perhaps the differences can be explained because our patients were hyperimmunised *in vivo*.

We conclude that cellular and humoral immune responses may develop *in vivo* in the absence of HL-A-linked MLR

differences and detectable MLR responses. These responses can be detected by lymphocyte-dependent antibody and by the existence of cytotoxic lymphocytes, both of which react with non-HL-A antigens. These minor histocompatibility differences can be detected *in vitro* by rapid ^{51}Cr release assays. Thus transplantation behaviour across minor histocompatibility antigens *in vivo* can now be correlated with an *in vitro* cytotoxic reaction.

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Pial vessels transport of substances from cerebrospinal fluid to blood

CEREBROSPINAL fluid (CSF) is formed in the choroid plexuses, in the ventricular cavities through ependymal walls¹ and in the subarachnoid space^{2,3}. Some authors^{3,4}, consider that subarachnoid fluid production constitutes 40% or more of the total secreted CSF. The formation of this subarachnoid fluid can be attributed to the pial vessels or be considered as a collection of extracellular fluid produced by the parenchymal capillaries, passing to the subarachnoid space through the pial membrane. Endothelial cells of the respective vessels should possess specific transport properties if they produce a fluid which might be of similar composition to that formed by the plexus. Absorption of the fluid and selective transfer of substances from CSF to blood should also be accomplished. The aim of this investigation was to explore which of the two, pial vessels or parenchymal capillaries, was the preferential route.

By implanting a plastic ring on the pial surface, it is possible to create an isolated subarachnoid reservoir where substances under study can be placed^{5,6}. By rapidly collecting successive samples from the sagittal sinus blood, it should be possible to study the time course of the passage of substances in different experimental conditions⁷. If communication between the subarachnoid cup and the venous pial vasculature were direct, the appearance of a substance in the sinus should be immediate, whereas if the route were from the subarachnoid cup to the brain tissue and then to the parenchymal capillaries, the appearance should be delayed and probably influenced by the varying degree of metabolism or binding of the substance with the tissue constituents.

Mongrel dogs, under nembutal anaesthesia (30 mg kg⁻¹,

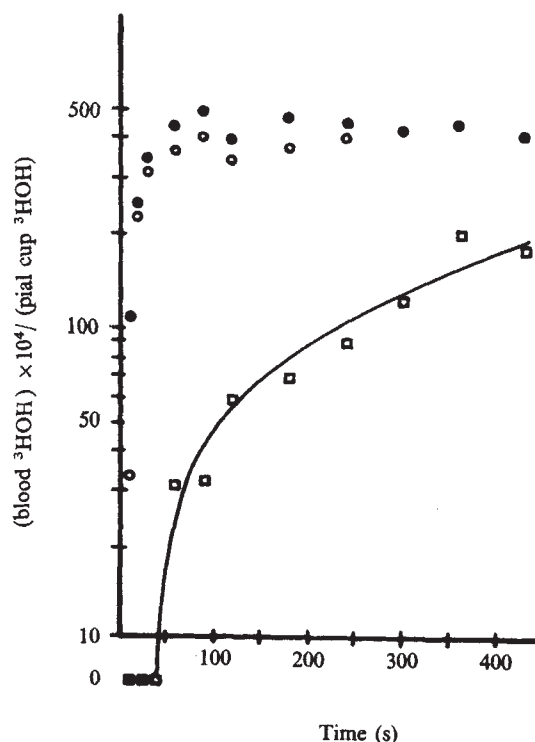


Fig. 1 Appearance of ³HOH in sagittal sinus blood. At zero time artificial CSF containing the isotope was poured into a subarachnoid cup and blood was collected in successive samples. ○ and ●, Two experimental runs in the same animal with the pia intact. A third run (□) was made after removing the pia, placing ³HOH on the naked cortex. In this case radioactivity was not detectable in the samples corresponding to the first 30–50 s. Applying a one-compartment analysis, the calculated *t*_{1/2} for the controls were 11 and 7 s, and for the run 'without pia', 347 s.

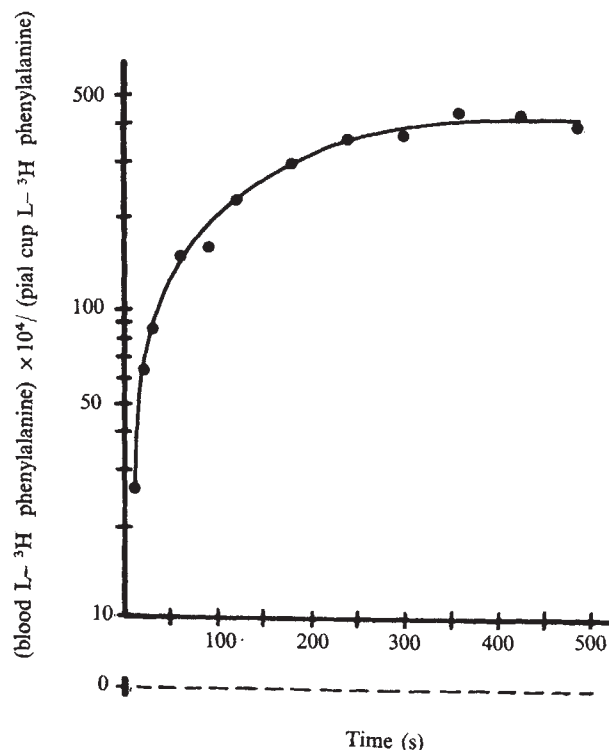


Fig. 2 Influence of removal of pia mater on the passage of L-³H-phenylalanine from a subarachnoid cup to blood. With the pia intact (—), the passage is relatively rapid (*t*_{1/2} = 92 s), whereas without pia (---) no significant radioactivity was found in blood throughout.

given intravenously) weighing 12 to 20 kg, were used throughout. For access to the sagittal sinus, a lucite adapter with a plastic cannula was tightly screwed into a hole drilled in the skull. The animal was heparinised to allow dropwise blood flow which could be interrupted by clamping the cannula. A plastic ring, 2 cm diameter, was placed on the pial surface of one of the hemispheres, the proximal border of the ring being 0.8 to 1.2 cm from the sinus adapter. The leak-proof plastic cylinder was kept in place by an agar gel without exerting pressure on the brain surface. Merlis artificial CSF⁸, pH 7.4, was used as a solvent for the tracer materials studied: ³HOH (water), L-³H-phenylalanine and ²²NaCl. The radioactivity concentration in the cup varied from 12 μCi ml⁻¹ (²²Na) to 25 μCi ml⁻¹ (³HOH). ²²Na was run simultaneously with the tritium labelled isotopes. L-³H-phenylalanine was directly diluted in Merlis CSF ('without carrier', 2 μM L-phenylalanine CSF). The artificial CSF containing isotopes were poured into the cup and thereafter collection of sinus blood in successive tubes was made at intervals varying from 1 to 60 s. When the lapse was longer than 10 s, blood was collected only the last 10 s of the interval. The longest experiment lasted 10 min. Blood and cup samples were deproteinised with absolute ethanol and after centrifugation an aliquot of the supernatant was used for radioactivity counting in a liquid scintillation spectrometer⁷.

The results were expressed as blood to cup radioactivity concentration ratios against time, as shown in Figs. 1 and 2. Several experiments were performed in each animal, with a rest interval of at least 30 min between runs. Because of the varying characteristics of pial vessel surface in the animal preparation, blood flow, and so on, it is more convenient to introduce experimental changes in the same animal where adequate controls could be run. Blood pressure in the femoral artery and flow from the sinus cannula were measured during the experiments as an index of normal circulation. Some animals showing brain oedema with protrusion of the cortex through the pial cup, were discarded.

TABLE 1 Compartmental analysis of the tracer appearance curves

³ HOH		L- ³ H-phenylalanine		²² Na
<i>t</i> _{1/2} (s)	<i>r</i>	<i>t</i> _{1/2} (s)	<i>r</i>	<i>t</i> _{1/2}
8	0.91 (9)	19	0.99 (7)	∞
5	0.99 (4)	14	0.97 (8)	∞
18	0.99 (10)	20	0.99 (11)	∞
20	0.99 (11)	22	0.96 (12)	∞
15	0.99 (14)	35	0.91 (13)	∞
11	0.99 (3)	92	0.99 (7)	∞
7	0.99 (3)			
28	0.99 (4)			
14	0.99 (4)			

Results of the linear least square regression fit for each experiment are given as $t_{1/2} = 0.693/\lambda$ (see text). The correlation coefficient *r* is followed by the number of experimental points in parenthesis. For ²²Na *t*_{1/2} was considered infinity as no blood radioactivity was detected during the experimental time. Each result corresponds to one animal.

The rate of ³HOH appearance in the sinus blood is fast during the first 30–50 s as shown in Fig. 1 ('with pia') probably indicating the passage of ³HOH through the pial vessels from the cup to the pia and then to the blood. ²²Na placed simultaneously in the cup did not appear at all in the venous blood, indicating that pial vasculature is not permeable to Na⁺. These results are similar to those obtained with the same cation introducing the tracer in the carotid⁷. The presence in parenchymal capillaries of a barrier impermeable to sodium but highly permeable to water, is indicated.

The interpretation that the way of transport is through pial vessels during the rapid ascending part of the curve in Fig. 1 ('with pia') is reinforced by experiments in which the pia mater was removed after application of AgNO₃ 5% solution for 5 min. It has been demonstrated that such a procedure does not affect the viability of the underlying tissue⁶. With the cup on the naked cortex another ³HOH run was performed (Fig. 1, 'without pia'). No ³HOH appeared in the venous blood until the 60 s sample. In the absence of the pial vessels the only route available after 60 s would be from the cup to the brain tissue and then to the blood.

Compartmental analysis of the data was performed assuming a one-compartment system (pia or brain tissue) with a constant input concentration which is the CSF in the pial cup. It can be seen in Fig. 1 that, as expected in this model, the blood concentration, *b*, raises approaching a plateau value, *b*_{max}. Therefore, a linear least-square fit of the experimental data was made according to the equation: $\ln(1 - b/b_{\max}) = -\lambda t$. For data 'with pia' in Fig. 1, *t*_{1/2} of 11 and 7 s were calculated with a correlation coefficient *r* of 0.99 for both. For the run 'without pia', *b*_{max} was assumed to be equal to that in the control experiments and the estimated *t*_{1/2} was 347 s, *r* = 0.95. All experiments were analysed in the same way, showing high correlation coefficients. The data are summarised in Table 1. The *t*_{1/2} for ³HOH was between 5 and 28 s, with an average of 14 s. For ²²Na experiments in which no radioactivity could be detected, the *t*_{1/2} was considered to be infinity.

L-Phenylalanine, an amino acid with high rate of passage through the parenchymal capillaries when introduced through

the carotid in the dog⁹ or rat¹⁰ was chosen to investigate the passage from the subarachnoid space to the blood. Figure 2 shows a typical curve of blood appearance of L-³H-phenylalanine 'without carrier' (2 μM L-phenylalanine in the cup). The curve is similar to that of ³HOH. When in the same animal the pia is removed and L-³H-phenylalanine 'without carrier' was placed on the naked cortex, no significant radioactivity was detected in sagittal sinus blood during the 8 min of the experiment. Results of the compartmental analysis of the L-³H-phenylalanine experiments 'with pia' are given in Table 1. As for ³HOH, a high correlation coefficient was found. The *t*_{1/2} values for five experiments averaged 22 s and in a sixth experiment *t*_{1/2} was 92 s.

The possible transport saturation of L-phenylalanine was tested comparing the rate of appearance of L-³H-phenylalanine in sinus blood, when the cup contained ³H-phenylalanine without carrier and 20 mM L-phenylalanine. These experiments are presented in Table 2. An inhibitory effect of about 50% is apparent, indicating that a mediated transport system is involved. A similar saturation phenomenon for disappearance of L-leucine from the subarachnoid fluid has been reported¹¹. In dog 1, the L-³H-phenylalanine passage in the first 60 s is higher for 20 mM L-phenylalanine than in the experiment 'without carrier'. In this animal, the 20 mM experiment was performed after the control, whereas the opposite order was used in dogs 2 and 3. It is possible that labelled L-phenylalanine incorporated in the brain after the first assay, exchanges (or counter-transport), with the cold L-phenylalanine of the second run, increasing the amount of label appearing in the sinus. From 120 s on, the inhibitory effect is evident.

Our results point to the participation of pial vessels in the transfer of substances from the subarachnoid CSF to blood. This transfer is selective for some substances, water, L-phenylalanine, and not for sodium for example. The transport is susceptible to saturation by the substrate in the case of the amino acid. These results are similar to the results of the experiments on dog brain where the molecules were introduced through the blood^{7,9}. Sodium, like sucrose, remains in the vascular space in a single passage through the brain circulation, whereas water is extracted from the blood almost completely⁷. L-phenylalanine, L-leucine and other neutral amino acids cross the blood-brain barrier by a mediated transport mechanism⁹. We suggest that endothelial cells of both pial vessels and tissue capillaries can maintain the differential composition of the CSF and blood.

There are several references pointing to the permeability of pial vessels to different substances^{12,13} and to their potential ability to form and to reabsorb CSF¹⁴. At least morphologically, the secretory conformation of choroid plexuses is not necessary for the formation of fluid. Perhaps their main function would be the active clearance of substances from CSF, the production and regulation of the fluid composition being reserved to endothelial cells of all the cerebral vasculature: pial vessels, parenchymal capillaries and choroidal capillaries.

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TABLE 2 Influence of carrier L-phenylalanine (20 mM) on the passage of L-(³H)phenylalanine from subarachnoid cup to blood

Dog	30 s	60 s	120 s	240 s	420 s
1	133	95	60	48	43
2	41	40	38	46	50
3	43	58	64	61	42

Three animals were used. The values listed are % control radioactivity calculated as blood-to-cup ratio in the 20 mM run divided by that in the run 'without carrier'. Both runs in the same animal. Times are intervals elapsed since the labelled solution was placed in the pial cup.

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Replacement of acidic phospholipids by acidic glycolipids in *Pseudomonas diminuta*

PHOSPHOLIPIDS are characteristic components of the membranes of living cells, where in many cases they represent the major polar lipid components. The common phospholipids in Gram-negative bacteria are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol¹. *Pseudomonas diminuta* NCTC 8545, however, contains high proportions of glycolipids and relatively low proportions of phospholipids when grown on solid media or in submerged culture². The glycolipids are α -glucosyldiglyceride, α -glucuronosyldiglyceride and β -glucosyl (1 $\rightarrow$ 4)- α -glucuronosyldiglyceride³, and the phospholipids are phosphatidylglycerol³ and 6-phosphatidyl- α -glucosyldiglyceride⁴. It has been suggested that the acidic glycolipids (containing glucuronosyl residues) might behave as substitutes for acidic phospholipids in the membranes of this pseudomonad⁵. Here we describe studies on the lipid composition of *P. diminuta* grown in continuous culture in conditions of phosphate and magnesium limitation which support this suggestion. Moreover, in conditions of phosphate limitation the cells contain barely detectable amounts (0.3% of total polar lipids) of phospholipid, thereby suggesting that appreciable amounts of these compounds are not obligatory for the maintenance of membrane function.

Continuous cultures of *P. diminuta* were prepared aerobically at 30°C in a 600 ml chemostat constructed essentially as described by Baker⁶; the pH was automatically controlled at 7.0 $\pm$ 0.2 and a dilution rate of 0.2 h⁻¹ was employed. Media were prepared using a standard mineral base⁷ with the addition of cystine (50 mg/l) and 1 ml/l of a solution of vitamins (40 mg each of pantothenic acid, biotin and vitamin B₁₂ in 100 ml) as recommended previously⁸; the carbon source was ammonium acetate (4 g/l). Phosphate-limited media contained KH₂PO₄ (25 mg/l) as the only phosphate-containing component and magnesium-limited media contained MgSO₄ (8 mg/l). Samples (250 ml) of steady state cultures were withdrawn from the chemostat vessel; the cells were collected by centrifugation, washed with NaCl (0.85%) and freeze dried.

Lipids were extracted from dry cells with chloroform-methanol (2:1, v/v) and the polar lipids examined by two-dimensional thin-layer chromatography followed by densitometry^{9,10}. The proportions of the polar lipids obtained from representative cultures of phosphate- and magnesium-limited chemostat growths are shown in Table 1. The chemical nature of the individual lipids was indicated by their reaction with specific spray reagents for lipid phosphates¹¹ and α -glycols¹²; the identity of each lipid was confirmed by chromatographic isolation and analysis of the products of hydrolysis as des-

cribed previously^{3,4}. In addition to the expected polar lipids, a small amount of an unidentified component was detected; its staining properties on thin-layer chromatograms indicated that it was a glycolipid.

The amounts of phospholipids in the phosphate-limited culture were extremely small; only a trace (maximum 0.3%) of phosphatidylglycerol was detected and phosphatidylglucosyldiglyceride was absent. The lipids of the magnesium-limited culture contained significant proportions of phospholipids, the overall lipid composition being similar to that of batch cultures grown in a complex medium (Oxoid Nutrient Broth No. 2). The greatly reduced proportion of phospholipids in

Table 1 Relative proportions of individual polar lipids in phosphate- and magnesium-limited chemostat cultures of *Pseudomonas diminuta* (expressed as % of the total polar lipid).

	Growth limitation	
	PO ₄ ³⁻	Mg ²⁺
Glucuronosyl diglyceride	33.0	27.0
Glucosylglucuronosyl diglyceride	24.0	21.0
Phosphatidylglycerol	0.3	22.0
Phosphatidylglucosyl diglyceride	—	7.0
Glucosyl diglyceride	40.3	18.0
Unknown glycolipid	2.4	5.0

cells grown in phosphate limitation compared with those grown in magnesium limitation supports Wilkinson's suggestion that acidic glycolipids might have a similar function to acidic phospholipids in the membranes of some bacteria. This suggestion was also supported by the discovery⁹ that in batches of *Bacillus cereus* T acidic phospholipids are partially replaced by an acidic glycolipid under certain growth conditions.

P. diminuta is unusual in several respects; it can be distinguished clearly by DNA homology studies from other pseudomonads¹³. It has relatively smaller proportions of phosphates in its cell envelope and is remarkable in that it does not synthesise phosphatidylethanolamine, an almost universal component of the membranes of Gram-negative bacteria¹. It is possible that glucosyldiglyceride may behave as a substitute for phosphatidylethanolamine in this organism as an interrelation between this phospholipid and diglucosyldiglyceride has been established in the lipids of Gram-positive bacilli^{9,14,15}. Diphosphatidylglycerol is not synthesised by *P. diminuta*, *P. pavonacea* and *P. rubescens*². In at least the first organism, phosphatidylglucosyldiglyceride might assume the function of diphosphatidylglycerol; both of these lipids contain four fatty acid residues and thus show a superficial structural similarity.

It is apparent, therefore, that the presence of phospholipids as major components of at least some biological membranes is not obligatory provided that other polar lipids, such as acidic glycolipids, are present. It is interesting that an acidic glycolipid, glucosyloxyoctadecenoic acid, has been found¹⁶ to comprise a much higher proportion (6%) of the total lipids of *Aspergillus niger* than does the sum of all the phospholipids (2%).

These results suggest limited interchangeability between polar lipid types within substitution classes¹⁷ which are essentially based on the overall charge of the lipids. Acidic phospholipids and acidic glycolipids form a major substitution group and zwitterionic phospholipids, ornithine amides¹⁸ and neutral glycolipids form another.

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530 nm) was measured, mainly from the middle and lower parts of the roots, by means of a quantometer apparatus. Experimental details will be described in full later.

When air was bubbling through the solution, a UG of the root was seen but when nitrogen was bubbled through the solution the intensity of the UG began to decrease sharply and after 2 h had practically ceased (Fig. 1). Stopping the passage of nitrogen failed to change the picture even though the shoots remained in air. Only the repeated bubbling of air through the solution led to a rapid increase of the roots' UG.

It is worth noting that other investigators have demonstrated that in the pumpkin¹² and other mesophytes^{13,14}, oxygen is not only transported from the parts of the plant above the ground to the roots but also partially flows out from the roots to the environment. Further studies of the factors responsible for these discrepancies are being made.

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New approach to study of oxygen transport in plants using chemiluminescent method

THE possibility that roots are aerated by molecular oxygen transported from the shoots continues to attract attention. A number of methods have already been involved in the study of this phenomenon, namely, polarography^{1,2}, labelled oxygen^{3,4}, enzyme investigations^{5,6}, and electron microscopy⁷⁻⁹. Nevertheless, the physiological role of this factor in the oxygen regime of the plant's roots remains unresolved and the data obtained in different laboratories vary.

Here we introduce a chemiluminescent method for tackling this problem. The phenomenon of an ultraweak glow (UG), or biochemiluminescence, discovered by Italian scientists¹⁰, takes place in plant and animal tissues only in the presence of oxygen¹¹. We chose this glowing of the roots, or the lack of it in anaerobic conditions, as a test for the transport of oxygen from the shoots to the radical zone of the pumpkin.

The roots of 25 pumpkin seedlings, 4-5 d old with expanded cotyledons, were submerged in Knop's solution. They were about 7-8 cm long and the solution level came to about 0.5-1 cm above the point of root attachment to the shoot—the rest of the shoot remaining in air. The intensity of the root UG (450-650 nm with a maximum of

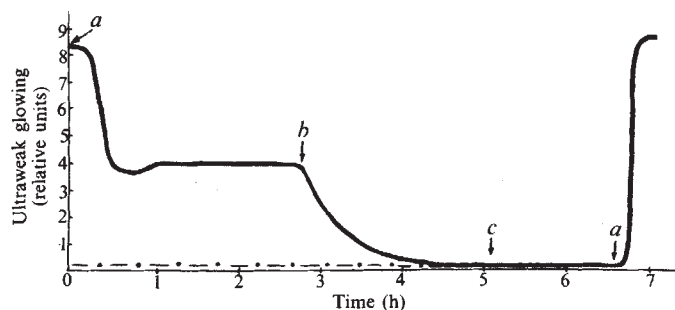


Fig. 1 Ultraweak glowing of pumpkin roots in different conditions of radical zone aeration. a, Bubbling by air; b, bubbling by N₂; c, stopping bubbling by N₂. o-o-o-o, Noise of apparatus (background).

Cell-free studies of developmental changes in synthesis of α -foetoprotein and albumin in the mouse liver

ALPHA foetoprotein (α FP) is an α -globulin present in foetal and early postnatal sera of various animal species. It is not normally detectable in adult serum by the immunodiffusion test, but in several pathological conditions, particularly primary hepatomas, the level of α FP increases to a significant level. The potential use of this phenomenon in early diagnosis of hepatomas has been explored. These and related studies on α FP and other 'foetal-tumour antigens' have been reviewed recently¹⁻⁵.

In addition to the possible clinical application, α FP may provide an unique opportunity for the understanding of the relationship between embryonic development and malignant transformation. It has been shown that α FP is synthesised in the yolk sac and liver, but the basic mechanisms for the synthesis and its regulation are essentially unknown. This

problem would best be approached by the use of cell-free systems in which all the requirements for the synthesis of α FP can be analysed.

As an initial step toward this goal, we attempted the synthesis of α FP in cell-free liver extracts derived from foetal and postnatal mice. We describe here that such extracts can produce α FP and albumin in relative amounts which vary depending on the stage of development of mice from which the liver extract was prepared.

Foetuses were removed from Swiss albino mice by caesarean section and killed by decapitation. The liver was quickly removed and ground manually in a glass homogeniser in 20 mM Tris-HCl buffer, pH 7.8, containing 5 mM MgCl₂, 100 mM KCl, 6 mM β -mercaptoethanol, 0.5 mM dithioerythritol (DTE), 1 mM EDTA and 0.25 M sucrose. The homogenate was centrifuged at 3,000g for 15 min and the

Polyacrylamide gel electrophoretic analysis of proteins synthesised by 18 d foetal liver extract and subsequently released by sonication is shown in Fig. 1a. Two main radioactive fractions were observed which showed slightly lower mobility than carrier α FP and albumin. To identify these proteins the sample was divided into two portions; one received antiserum against α FP and the other antiserum against adult mouse serum (to precipitate albumin). These antisera possessed the required specificity to α FP and adult serum proteins as tested by double immunodiffusion and immunoelectrophoresis.

After incubation, antigen-antibody precipitates were removed and the supernatant containing proteins not bound to antibody was analysed by polyacrylamide gel electrophoresis. In the sample which received antiserum against α FP the faster-moving fraction remained as the major radioactive peak (Fig. 1b). In the other sample which received antiserum against adult mouse serum, the slower moving fraction remained as the major radioactive peak (Fig. 1c). This indicated that the faster moving radioactive protein was albumin and the slower moving protein α FP.

Mouse α FP has been shown to exhibit microheterogeneity or several electrophoretic forms due to a variation in the amount of sialic acid residues⁶. As the sialation process is distinct from the synthesis of polypeptide chains it is possible that α FP produced in the cell-free system was not fully sialated and thus exhibited a lower electrophoretic mobility

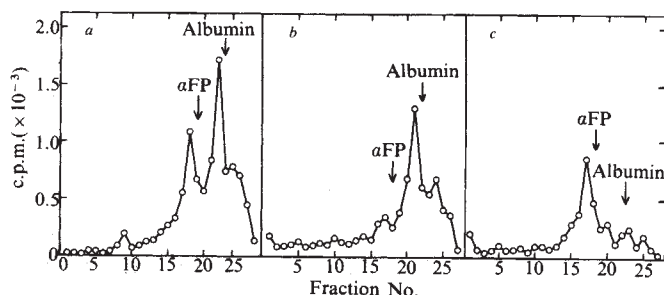


Fig. 1 Polyacrylamide gel electrophoresis of α FP and albumin produced by foetal liver lysate and their identification by immunoprecipitation. *a*, A lysate of 18 d-old foetal liver was incubated with 20 μ Ci each of ³H-leucine (1 Ci mmol⁻¹), ³H-valine (2 Ci mmol⁻¹) and ³H-phenylalanine (6.15 Ci mmol⁻¹) (Amersham/Searle, Oakville, Ontario), 0.025 μ mol each of 17 other amino acids, 1 μ mol ATP, 0.3 μ mol GTP, 10 μ mol phosphocreatine, and 50 μ g creatine phosphokinase ml⁻¹ at 30°C for 60 min. At the end of incubation the reaction mixture was centrifuged at 149,000g for 90 min. The pellet was resuspended in water, frozen and thawed and then sonically disrupted in a Bronson Sonifier at setting 5 for 5 min. This was then centrifuged at 149,000g for 90 min and the supernatant was analysed by polyacrylamide gel electrophoresis (7% gel, pH 8.9) according to Davis¹². The gel was stained with Amido Black, scanned at 650 m μ and then sliced into 2 mm fractions which were dissolved in 30% H₂O₂ at 60°C overnight. Radioactivity was measured using Aquasol scintillation liquid (NEN Canada, Dorval, Quebec). *b*, The sample shown in *a* was incubated with rabbit antiserum against mouse α FP at 37°C for 60 min and then at 0°C overnight. The precipitate was removed by centrifugation and the supernatant analysed by polyacrylamide gel electrophoresis. *c*, The sample shown in *a* was incubated with rabbit antiserum against adult mouse serum (Nutritional Biochemical Corporation, Cleveland, Ohio) as described in *b*. The precipitate was removed by centrifugation and the supernatant analysed by polyacrylamide gel electrophoresis. Arrows indicate the carrier α FP and albumin bands.

supernatant was applied to a Sephadex G-25 column in 20 mM Tris-HCl, pH 7.8, containing 4 mM MgCl₂, 55 mM KCl, 6 mM β -mercaptoethanol, 0.5 mM DTE and 1 mM EDTA. The void volume fraction was incubated with radioactive amino acids and other components necessary for protein synthesis as described in the legend of Fig. 1.

There was active protein synthesis in these conditions as measured by hot trichloroacetic acid-precipitable counts. Preliminary analysis showed, however, that newly-synthesised radioactive α FP was not released free into the supernatant. This suggested that α FP was synthesised by membrane-bound polysomes and the completed polypeptide chains remained bound to the membrane or in microsomal cisternae. Several treatments were tested to disrupt the membrane structure. A combination of freeze-thawing and sonication was found to be among the most effective for this purpose. This procedure also permits the release of newly formed albumin from inside the microsomal cisternae.

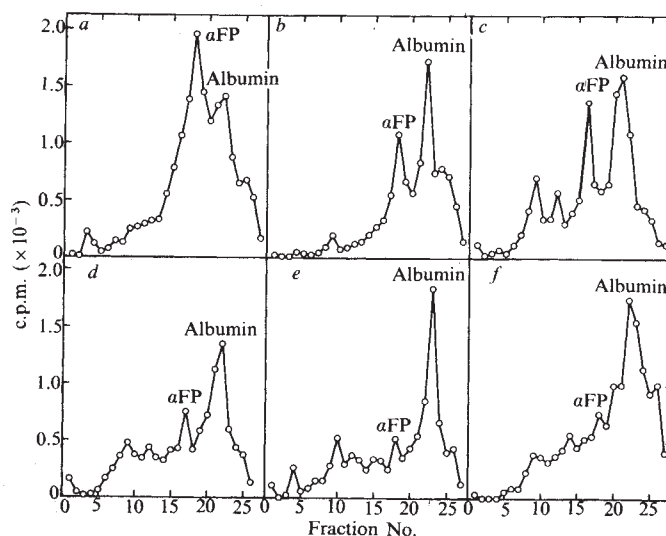


Fig. 2 Polyacrylamide gel electrophoresis of proteins produced by liver lysates from foetal and postnatal mice. Conditions for cell-free protein synthesis and gel analysis are described in the legend of Fig. 1. *a*, 13-d-old foetus; *b*, 17-d-old foetus; *c*, 20-d-old foetus; *d*, 1-d-old mice; *e*, 3-d-old mice; *f*, 6-d-old mice.

than carrier α FP. The exact cause for the slightly slower migration of radioactive albumin than carrier albumin is not clear at present, but a similar observation has been reported by others⁷.

Figure 2 shows the synthesis of α FP and albumin in liver extracts from developing mice ranging in age from 13 d of gestation to 6 d after birth. It is seen that the synthesis of α FP decreased with time, whereas that of albumin increased. We found that, although the total radioactive proteins released by sonication varied from experiment to experiment, the % of α FP and albumin and their relative amounts remained relatively constant. The molecular weight of mouse α FP, as estimated by sodium dodecyl sulphate (SDS) gel electrophoresis, was 70,000, which was similar to that of mouse albumin (unpublished data). The radioactivity in the α FP and albumin fractions shown in Fig. 2 is, therefore,

directly proportional to the total amounts synthesised in the cell-free extracts.

The changes in the synthesis of α FP and albumin shown above were calculated as relative amounts and are presented in Fig. 3. In the 13-d-old foetus, the synthesis of α FP was twice as active as that of albumin. The subsequent decrease in α FP production and increase in albumin synthesis showed that at about day 15 of gestation they were produced at about an equal rate. By the time the mice were born the α FP:albumin ratio decreased to less than 1:2. After birth, the synthesis of α FP continued to decline and seemed to cease after about one week as analysed by the present method.

The level of albumin synthesis was remarkably high in the foetus of 13 d gestation. Analysis of the liver in the earlier gestation was not feasible because of its small size.

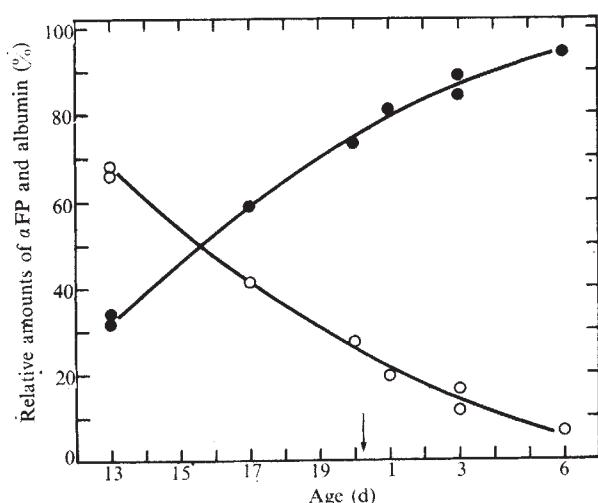


Fig. 3 Relative amounts of α FP and albumin synthesised by liver lysates from foetal and postnatal mice. The sum of the counts under α FP and albumin peaks in Fig. 2 was used as 100%. O, α FP; ●, albumin. Arrow indicates birth.

The results described above show that the present cell-free system enables the analysis of relative synthetic activity for α FP synthesis and albumin production in the liver at various stages of development. The observed shift in the synthetic activities with maturation is in agreement with the changes in their total serum contents based on immunological analysis⁸. It is likely, therefore, that the present cell-free synthesis of α FP and albumin reflects the pattern of protein synthesis operative *in vivo*. A similar approach can be applicable to other mammalian species, and may prove to be of value as an aid in diagnosis of hepatomas.

The present study suggests that mRNA for α FP may be isolated from foetal liver and translated in a defined cell-free system. In fact, the study in progress has strongly supported this possibility. The synthesis of albumin using exogenous mRNA has recently been accomplished⁹⁻¹¹. The study of requirements for the translation of these mRNAs, such as initiation factors, should help us to understand the molecular basis for the regulation of the α FP and albumin synthesis during embryonic development. The knowledge gained in such studies may also provide a basis for possible engineering of the synthetic events in tumour cells to arrest or reverse neoplastic transformation.

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Blocking of syngeneic effector T cells by soluble tumour antigens

THE cellular immune response to murine sarcoma virus (MSV)-induced tumours in mice involves lymphoid effector cells of different origins^{1,2}. When the chromium release test (CRT)³⁻⁵ is used to study this immune response, only thymus-derived lymphoid cells (T cells) show any appreciable lytic activity towards the appropriate tumour target cells^{6,7}. Other lymphoid cells display no lytic activity in CRT, nor are they required for T cell-dependent tumour cell lysis to occur⁷. When the microcytotoxicity assay (MA) of Takasugi and Klein⁸ is used to study the cellular immune response of the same MSV tumour-bearing mice, both T and non-T lymphoid cells are seen to be active^{1,2}.

A time-course study with these two assays using the same lymphoid cell preparations¹ showed that, at the moment of maximum tumour size, neither T nor non-T lymphoid cell activity was detected in MA, whereas a peak value of T cell activity was detected in CRT. It has also been shown that sera from MSV tumour-bearing mice (progressor sera) are able to completely inhibit the immune activity measured in MA^{1,9}, although the same sera have no effect when tested in CRT^{1,4,6}. These facts indicated that a different cytotoxic T cell subpopulation might be involved in each test, the effector T cells active in MA probably being blocked by the soluble tumour antigens present in progressor sera^{9,10} and the effector cells active in CRT not being at all affected. Here we present further arguments in favour of such an hypothesis.

We tested the effect of progressor sera or soluble tumour-cell antigen extracts on the immune activity of spleen cells from MSV-infected C57BL/6 (B6) mice, as revealed by MA and CRT. The Moloney isolate of MSV, regularly maintained by acellular transmission in newborn B6-mice, was used in all experiments to induce experimental sarcomas in adult B6 mice. Spleen cells were taken between the 15th and 25th days after MSV inoculation because the T cell subpopulation shows an appreciable immune activity in both MA and CRT during this period^{1,2}, and the tumour has regressed. CRT and MA were performed as previously described^{1,4}, and syngeneic conditions were maintained throughout all experiments. Spleen lymphocytes

from normal B6 mice were used as negative controls in all tests.

The experiments summarised in Table 1 investigated the blocking activity of B6 progressor serum (PrSm) on spleen effector cells. The cytostatic activity detected in MA could be blocked at a limited range of serum dilutions (1/6-1/12), while no effect was evident in CRT with the same lymphocyte samples and serum dilutions. Normal B6 serum (N1 Sm), at similar dilutions, did not affect the lymphocyte activity in either test.

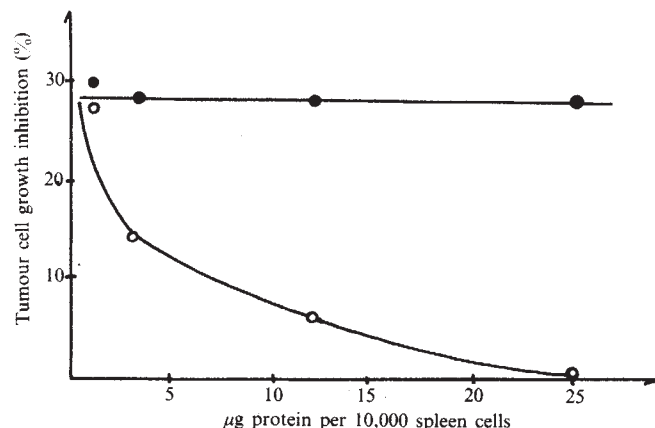


Fig. 1 The effect of GiL4 antigen (O) and of normal B6 antigen (●) on the immune activity, as detected in MA, of spleen cells from B6 mice infected with MSV 20 d previously.

Baldwin *et al.*¹¹ have shown that the blocking fraction of serum from tumour-bearing rats contains antigen-antibody complexes, and that solubilised tumour antigen seems to be the main blocking agent^{10,11}. Sjögren *et al.*¹² had previously reached the same conclusion concerning the blocking factor in sera from MSV-infected mice. We therefore solubilised tumour cell antigens and checked whether they could block specifically the spleen cell activity detected in MA and in CRT.

The procedure developed by Mettzer *et al.*¹³ and Reisfeld *et al.*¹⁴ was chosen; this involves antigen solubilisation by 3 M KCl, pH 7.4, and gives high yields of functional antigen. Soluble antigens were prepared from normal spleen cells or from several different tumour cell lines belonging to the FMRGi antigen group¹⁵ (MSV-tumour cells are FMRGi-positive). Normal or immune spleen lymphocytes were incubated for 18 h (CRT) or 48 h (MA) in the presence of target tumour cells and of antigen extract. As shown in Table 2 the cytostatic activity of immune spleen cells in MA was inhibited by the tumour antigen extracts prepared from GiL₄, MSV-tumour, or Friend leukaemia cells. No blocking effect was detected in CRT at equivalent antigen doses; indeed, in some cases immune lysis actually increased in CRT in the presence of soluble antigen.

Inhibition by the soluble antigen preparations in MA is specific because: first, the soluble extracts prepared from normal B6 or BALB/c spleen cells do not block the cytostatic activity of anti-MSV tumour lymphocytes at any of the doses used; second, when an allogeneic immune system is studied in MA, the B6 'normal' extract blocks BALB/c lymphocytes (H2d) immunised against B6 normal spleen cells (H2b) (Table 3); and third, the BALB/c soluble extract prepared from Friend virus-induced leukaemia cells blocks the activity in MA of B6 lymphocytes immunised against an MSV-induced tumour (Table 2), but it does not affect the activity in MA of BALB/c anti-B6 lymphocytes (Table 3). This also indicates that the inhibitory activity of our antigen extracts is not the result of a toxic effect on spleen cells.

The blocking effect displayed by the soluble antigen extracts in MA follows a dose-response curve (Fig. 1). The extract prepared from GiL₄ cells blocks lymphocyte activity completely at doses equivalent to or higher than 25 µg protein

per 10,000 spleen cells; blocking activity can still be detected at 3 µg protein per 10,000 spleen cells, but disappears at the 1 µg protein level.

The experiments discussed above show that the immune cytostatic activity of lymphoid cells in MA can be blocked when functional T and non-T effector cells are present^{1,2}. As the effector cells involved in CRT are exclusively T cells^{4,6,7} and cannot be blocked, it seemed important to determine if the effector T cells active in MA, when purified, could be blocked or not. Cell populations enriched in T cells were consequently obtained by passing whole spleen cell suspensions through anti-immunoglobulin coated glass bead columns^{1,16} and were subsequently used to study the blocking effects of progressor sera and of soluble antigen extracts. Table 4 shows that column-purified cells can be effectively blocked in MA when they are incubated with progressor serum or with the adequate soluble tumour extract. No decrease in immune lysis is detected in CRT when the same lymphocytes are incubated with these reagents at comparative doses.

Our column-purified cell preparations contained $89 \pm 2\%$ θ_{CH} positive cells and $1 \pm 0.5\%$ immunoglobulin-positive cells⁷. To study the role played in MA by the θ_{CH} negative cells present in the column-purified cell suspensions, column-passed cells were treated, with AKR anti- θ_{CH} serum and rabbit complement and dead cells were removed by incubation with 0.05% trypsin. A pure population of viable column-passed cells not carrying the θ_{CH} antigen was thus obtained and was adjusted to the appropriate cell number before distribution in the MA plates. These spleen cells possessed no ability to inhibit tumour cell growth; experimental results published recently^{1,2} show that non-T cells do not require a T cell collaboration to exert a cytostatic effect in MA. Control cells trypsinised in the same manner after incubation with normal AKR serum and complement showed no decrease in their immune cytostatic activity. Consequently, the immune activity displayed by such column-purified cells in MA seems to be due exclusively to thymus-dependent lymphocytes.

In a different set of experiments, cytostatic non-T cells were purified by treating whole spleen cell preparations with anti- θ_{CH} serum and complement. The immune activity of 10,000 anti-MSV non-T cells was completely inhibited in MA by B6 progressor serum diluted 1/6 or by 25 µg GiL₄ soluble antigen. Normal B6 serum and normal B6 antigen had no effect on the immune activity of these cells.

Our results provide further proof of the hypothesis that CRT and MA, as performed here, do not measure the same immune phenomena. As suggested in a recent communication¹, CRT reveals an exclusively T cell-mediated cytotoxicity, while MA seems to detect a complex cytostatic effect in our experimental conditions. This segregation of effector cell functions is probably because the target cell used in CRT is highly resistant to immune cytotoxicity, while the target cell used in MA is easily lysed¹. We have provided evidence here which shows that soluble antigens prepared from MSV-induced tumours or from antigenically related tumours can block the activity of anti-MSV effector T cells in MA, whereas no inhibition of the effector T cells active in CRT can be detected. The possibility of blocking immune activity in MA with soluble tumour antigen preparations has already been indicated by Baldwin *et al.*^{10,11} in other tumour systems. C. Neauport (unpublished) has recently obtained results in our laboratory showing that the activity of anti-allogeneic immune lymphocytes in CRT is very difficult to block with soluble antigen preparations, differing with results published by Wagner and Boyle¹⁷ and agreeing with previous observations of Cerottini *et al.*¹⁸. We have also provided evidence that progressor sera can only block the activity of effector T cells in MA and not in CRT, thus confirming previous observations^{1,4,9}. This blocking effect could result from the presence of soluble tumour antigens in the serum of progressor mice, and it might explain why no activity can be detected in MA when lymphocytes are taken at the time of maximum tumour size¹. The fact that the effector T cells de-

Table 1 Effect of B6 progressor serum on spleen cell immune activity.

Days after MSV inoculation	Cells incubated with:	MA			Degree of blocking (%)	CRT Immune cytotoxicity (%)
		Normal spleen cells	Immune spleen cells	Tumour cell growth inhibition (%)		
15	MEM	199 ± 25	148 ± 18	26*	54†	24.0 ± 3.8
	NIsm (1/6)	205 ± 29	168 ± 26	18‡		24.1 ± 3.5
	PrSm (1/6)	205 ± 19	188 ± 9	8‡		20.2 ± 3.9
19	MEM	229 ± 20	180 ± 21	21*	72*	17.8 ± 1.3
	NIsm (1/6)	224 ± 27	155 ± 21	31§		11.0 ± 1.5
	PrSm (1/6)	192 ± 50	173 ± 35	10‡		12.0 ± 3.3
	NIsm (1/12)	225 ± 26	183 ± 14	19*	100*	12.3 ± 2.6
	PrSm (1/12)	199 ± 33	197 ± 22	0		12.2 ± 3.7
	NIsm (1/18)	248 ± 36	205 ± 23	17†	0	14.9 ± 3.0
	PrSm (1/18)	248 ± 31	187 ± 28	24*		12.8 ± 3.4
	NIsm (1/24)	239 ± 22	184 ± 22	23*	0	Not done
	PrSm (1/24)	255 ± 43	204 ± 24	20†		
21	MEM	163 ± 24	119 ± 14	27§	100*	15.9 ± 3.6
	NIsm (1/6)	152 ± 23	122 ± 14	20*		11.7 ± 2.8
	PrSm (1/6)	160 ± 22	159 ± 26	0		18.5 ± 1.5
24	MEM	121 ± 17	76 ± 13	37§	75*	13.2 ± 3.1
	NIsm (1/6)	167 ± 17	127 ± 18	24*		13.9 ± 3.8
	PrSm (1/6)	180 ± 18	171 ± 10	5‡		13.8 ± 3.5

Normal or immune B6 spleen cells were incubated with normal B6 serum (NIsm) or B6 progressor serum (PrSm) for the duration of each test. In MA, 3034 Falcon Plastics tissue culture microplates were seeded with 100 to 200 B6 MSV tumour cells growing in monolayers and were incubated overnight; 5 µl Eagle's MEM, NIsm or PrSm plus 10,000 lymphocytes in 5 µl were then added, and the plates were further incubated at 37°C for 48 h. After one wash, the remaining cells were fixed with methanol, stained with giemsa and counted. The numbers shown represent the arithmetic mean of target cell counts ± s.d. and the significance of the relative inhibition of cell growth was calculated according to Student's *t* test. The degree of blocking in MA was evaluated by taking into consideration the difference between the number of target cells counted in the presence of immune spleen cells and of normal spleen cells (ΔTC) following incubation with progressor serum (ΔTC_{PrSm}) or with normal B6 serum (ΔTC_{NIsm}): Degree of blocking (%) = $(\Delta TC_{NIsm} - \Delta TC_{PrSm}) / (\Delta TC_{NIsm}) \times 100$.

In CRT 20,000 ⁵¹Cr-labelled B6 GiL4 ascitis lymphoma cells in 0.05 ml were mixed with 0.1 ml MEM, NIsm or PrSm, and 500,000 normal or immune B6 spleen cells in 0.05 ml were then added. After 18 h of incubation the reaction mixture was adjusted to 1.0 ml and percent chromium release was determined as described elsewhere⁷. The results shown represent the differences between chromium released in the presence of immune spleen cells and of normal spleen cells ± s.d., and are statistically significant. A low lymphocyte to target cell ratio (25:1) was chosen to give low levels of immune lysis which theoretically should be easily blocked. No blocking effects were detected with CRT in any of the samples studied.

**P* < 0.01

†*P* < 0.05

‡*P* > 0.05

§*P* < 0.001

Table 2 Effect of soluble antigen preparations on spleen cell immune activity.

Days after MSV inoculation	Cells incubated with:	MA			Degree of blocking (%)	CRT Immune cytotoxicity (%)
		Normal spleen cells	Immune spleen cells	Tumour cell growth inhibition (%)		
20	MEM	220 ± 20	163 ± 14	26*	100†	14.9 ± 3.8
	B6NIAg (25 µg)	234 ± 28	168 ± 13	28*		12.2 ± 3.2
	GiL4Ag (25 µg)	139 ± 14	138 ± 26	0		14.8 ± 3.6
21	MEM	354 ± 37	272 ± 26	23*	95†	14.1 ± 2.0
	B6NIAg (25 µg)	370 ± 39	271 ± 35	28*		12.7 ± 2.5
	GiL4Ag (25 µg)	327 ± 58	322 ± 54	2‡		19.6 ± 3.0
	B6NIAg (12 µg)	450 ± 24	348 ± 47	23*	100†	13.8 ± 2.2
	GiL4Ag (12 µg)	441 ± 61	454 ± 61	0		16.5 ± 1.4
	B6NIAg (12 µg)	412 ± 37	342 ± 31	17†	100†	13.8 ± 2.2
	B6MSVAg (12 µg)	348 ± 43	371 ± 69	—6‡		15.2 ± 1.8
	MEM	161 ± 17	131 ± 13	19*	85‡	13.1 ± 2.6
22	CNIAg (25 µg)	156 ± 10	116 ± 17	26*		12.8 ± 3.8
	FVLA (25 µg)	89 ± 12	83 ± 18	7‡		11.0 ± 3.5

Normal or immune spleen cells were incubated with each soluble antigen preparation during the whole duration of each test at the ratio of 25 or 12 µg protein for 10,000 lymphocytes. MA and CRT were performed as described in Table 1. Soluble antigens were prepared from B6 normal spleen cells (B6NIAg), BALB/c normal spleen cells (CNIAg), GiL4 lymphoma cells of B6 origin (GiL4Ag), solid B6 MSV-induced tumours (B6MSVAg), YC8 lymphoma cells of BALB/c origin (YC8 Ag), and Friend virus-induced leukaemia cells of BALB/c origin (FVL Ag). Following solubilisation in 3M KCL, pH 7.4, the crude soluble antigen preparations were filtered through Sephadex G-200 columns; the fractions presenting antigenic activity were detected by the specific inhibition of cytotoxic antisera in the presence of complement, and their protein content was evaluated with the Folin-Ciocalteu reagent.

**P* < 0.001

†*P* < 0.01

‡*P* > 0.05

Table 3 Effect of soluble antigen preparations on H2d anti-H2b immune spleen cells.

Cells incubated with:	MA			CRT	
	Normal spleen cells	Immune spleen cells	Tumour cell growth inhibition (%)	Degree of blocking (%)	Immune cytotoxicity (%)
MEM	142 ± 22	111 ± 8	22*	0	30.9 ± 3.1
CNI _{Ag} (25 µg)	126 ± 15	103 ± 11	18*	0	32.4 ± 3.5
B6NI _{Ag} (25 µg)	106 ± 11	97 ± 13	8†	61*	34.4 ± 3.9
FVLA _{Ag} (25 µg)	98 ± 4	64 ± 13	35‡	0	35.1 ± 3.1

Immune H2d anti-H2b spleen cells were obtained 6 d after inoculating adult BALB/c mice with 5×10^7 B6 normal spleen cells intraperitoneally. Target cells were the same as those used to test anti-MSV tumour immune lymphoid cells. Spleen cells from normal BALB/c mice were used as negative controls.

* $P < 0.01$

† $P > 0.05$

‡ $P < 0.001$

Table 4 Effect of progressor serum and soluble antigens on column-purified T cells.

Experiment	Days after MSV inoculation	Cells incubated with	MA			CRT	
			Normal spleen cells	Immune spleen cells	Tumour cell growth inhibition (%)	Degree of blocking (%)	Immune cytotoxicity (%)
1	20	MEM	152 ± 19	129 ± 16	15*	100‡	18.9 ± 1.1
		NI _{Sm} (1/6)	195 ± 35	162 ± 31	17*		19.7 ± 2.5
		Pr _{Sm} (1/6)	178 ± 20	182 ± 29	—2†		18.0 ± 3.8
2	20	MEM	122 ± 18	86 ± 19	30‡	82‡	10.6 ± 3.2
		B6NI _{Ag} (25 µg)	138 ± 12	104 ± 18	25§		13.0 ± 2.8
		GiL4 _{Ag} (25 µg)	111 ± 16	105 ± 20	5†		18.1 ± 3.5
3	21	MEM	170 ± 15	149 ± 13	12*	100‡	15.6 ± 3.3
		NI _{Sm} (1/6)	192 ± 18	169 ± 14	12*		14.3 ± 2.6
		Pr _{Sm} (1/6)	192 ± 14	201 ± 26	—5†		16.7 ± 3.0
4	21	MEM	221 ± 19	173 ± 14	22§	78‡	17.8 ± 1.8
		B6NI _{Ag} (25 µg)	259 ± 32	196 ± 25	24§		16.2 ± 2.3
		GiL4 _{Ag} (25 µg)	216 ± 35	202 ± 32	6†		24.8 ± 3.6

* $P < 0.05$

† $P > 0.05$

‡ $P < 0.01$

§ $P < 0.001$

tected in CRT are not sensitive to such blocking factors further indicates that two different effector T cell subpopulations are involved here. These two effector T-cell subpopulations probably differ in function as well as in sensitivity to blocking factors, since the subpopulation detected in CRT displays a cytolytic activity and the subpopulation detected in MA displays a cytostatic activity. These observations recall the functional heterogeneity of T cells detected in other immune systems¹⁹⁻²¹. It cannot be definitely excluded, however, that in our experimental system effector T cell subpopulations of similar nature are involved which react with different antigens of the tumour cell membrane.

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Allogeneic stimulation modulates the *in vitro* response of T cells to transplantation antigen

THE theory of allogeneic stimulation¹ postulates that transplantation antigens, such as H-2 in the mouse or H-LA in man, are not strong immunogens for allogeneic animals, but are rendered highly immunogenic when presented to the immune system in conjunction with an allogeneic stimulus. The capacity to provide an allogeneic stimulus is the property of metabolically active²⁻⁴ immunocompetent cells¹, and it has been suggested that allogeneic stimulation requires the transfer of some cytoplasmic component from the stimulating to the responsive cell¹. According to this hypothesis, any treatment that inactivates the metabolic activity of lymphoid cells will reduce their immunogenicity for other members of the same species.

A prediction of this hypothesis is that transplantation antigen alone, either soluble or cell bound, will be poorly immunogenic when presented to potentially responsive immunocompetent cells. If an allogeneic stimulus is provided at the same time, however, this second stimulus will activate the potentially reactive cell and facilitate its response to the test antigen. Here we show that lymphocytes inactivated with ultraviolet light are unable to stimulate allogeneic lymphocytes in mixed leukocyte culture (MLC), and do not generate a significant cell-mediated cytotoxic response in such cultures. Cytotoxic activity can, however, be generated if an allogeneic stimulus is provided by a third party, γ -irradiated lymphocyte that is antigenically different from the ultraviolet-irradiated cell.

MLC reactivity was assayed by mixing responsive lymph node cells (C57BL/6J) with γ - or ultraviolet-irradiated stimulating spleen cells (BALB/c) in Linbro multi-dish culture trays (Linbro FB 24-TC). The culture medium used was Eagle's Minimum Essential Medium (Grand Island Co., F15) containing penicillin (100 $\mu\text{g ml}^{-1}$), streptomycin (100 $\mu\text{g ml}^{-1}$) and neomycin (100 $\mu\text{g ml}^{-1}$). The medium was supplemented with 10% foetal calf serum and 2-mercaptoethanol to a final concentration of 10^{-4} M. Stimulating spleen cells, at a density of $1 \times 10^7 \text{ ml}^{-1}$, were incubated in culture medium for 1.5 h at 37°C before irradiation⁵. ^{60}Co γ -irradiation (800 r) was given at a dose rate of 280 r min^{-1} and ultraviolet irradiation was given by exposing 5 ml of cell suspension in open 80 mm Petri dishes for 4 min, at a distance of 20 cm from a 30 W germicidal ultraviolet lamp. Following irradiation, the cells were washed once and resuspended in culture medium.

To control the specificity of MLC activation, and any non-specific effects resulting from the addition of ultraviolet-irradiated cells to the test cultures, the following spleen cell mixtures were used as the stimulating population in MLC: γ -irradiated BALB/c + ultraviolet-irradiated C57BL/6J; ultraviolet-irradiated BALB/c + γ -irradiated C57BL/6J; and ultraviolet-irradiated C57BL/6J + γ -irradiated C57BL/6J. These stimulating cell populations were prepared by mixing equal volumes of each component at a cell density of $2 \times 10^6 \text{ ml}^{-1}$. In MLC assays the stimulating spleen cell population was mixed with an equal volume of C57BL/6J lymph node cells at a density of $1 \times 10^6 \text{ ml}^{-1}$. Each mixture (2.5 ml) was added to three replicate wells of Linbro culture trays and cultured for the required time at 37°C in an atmosphere of 10% CO_2 , 7% O_2 and 83% N_2 . Aliquots (0.2 ml) were taken from each culture over a 5 d period and added to Linbro 16 mm microtitre plates with 3 μCi of ^3H -thymidine (Amersham, TRA 120, specific activity, 5 Ci mmol^{-1}). Thymidine uptake was estimated 5 h later by collecting the cultured cells on glass fibre filter disks, using an automatic cell collecting device (MASH-11, Microbiological Associates), and counting the amount of thymidine incorporation in a liquid scintillation counter.

The kinetics of thymidine uptake are shown in Fig. 1. Ultraviolet irradiation of BALB/c spleen cells, in contrast to γ -irradiation, inactivates their capacity to provide an allo-

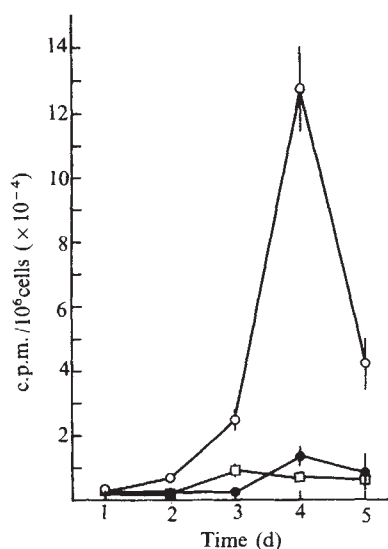


Fig. 1 Stimulation of ^3H -thymidine uptake by C57BL/6J lymph node cells in mixed cultures containing the following spleen cell populations: O, γ -irradiated BALB/c + ultraviolet-irradiated C57BL/6J; ●, ultraviolet-irradiated BALB/c + γ -irradiated C57BL/6J; □, γ -irradiated C57BL/6J + ultraviolet-irradiated C57BL/6J. ^3H -thymidine incorporation is expressed as c.p.m./ 10^6 lymph node cells and the vertical bars show the standard error of the mean of three replicate assays.

genic stimulus for C57BL/6J lymph node cells as measured by MLC activation. These findings confirm those of Lindahl-Kiessling *et al.*⁴. Moreover, ultraviolet irradiation did not affect the expression of alloantigen on the surface of irradiated cells (Table 1), as determined by antibody absorption. Antigenic activity was estimated by microcytotoxicity titration before and after absorption of alloantiserum with γ - or ultraviolet-irradiated BALB/c spleen cells.

The *in vitro* generation of cytotoxic lymphocytes was carried out in Falcon plastic tissue culture flasks (30 ml), using the MLC medium described above. Ultraviolet-irradiated BALB/c (H-2^d) spleen cells, either alone or mixed with γ -irradiated CBA/H (H-2^k) spleen cells, were used to stimulate C57BL/6J (H-2^b) lymph node cells *in vitro*. After 4 d, the cultures were examined microscopically and the blast cell content scored semiquantitatively. These cultures were then collected and

Table 1 Antibody binding capacity* of γ - and ultraviolet-irradiated BALB/c spleen cells

Absorbing spleen cell population (10^7 cells)	Cytotoxic titre
γ -irradiated BALB/c	1 : 10
Ultraviolet-irradiated BALB/c	1 : 10
γ -irradiated C57BL/6J	1 : 160
Ultraviolet-irradiated C57BL/6J	1 : 160

* Cytotoxic anti-BALB/c antibody was prepared in C57BL/6J mice. 0.5 ml of antiserum was absorbed with the different cell populations for 2 h at room temperature. Residual cytotoxic activity was assayed against BALB/c lymph node cells, in the presence of guinea pig complement. Antibody titrations were carried out with doubling dilutions of serum and the 50% cytotoxicity end point was estimated by trypan blue exclusion.

cytotoxic cells specific for the H-2^d antigen assayed against ^{51}Cr -labelled P815 mastocytoma (H-2^d) target cells⁶. Table 2 shows the detailed experimental protocol, including controls for specificity and any nonspecific effects of ultraviolet-irradiated cells, and the relative potencies of the resultant cytotoxic cell populations. Cytotoxic potency was obtained by plotting the logarithm of the number of target cells lysed against the logarithm of the number of sensitised cells used in the assay (Fig. 2). Such plots have a linear dose response relationship with unit slope to a level of approximately 35% specific lysis⁷.

Table 2 Relative cytotoxic potency and blast cell content of cultures following *in vitro* cultivation of C57BL/6J lymph node cells with different stimulating spleen cell populations

Composition and density of stimulating spleen cell population*		Blast cell content after 4 d in culture	Relative cytotoxic potency†
$8 \times 10^6 \text{ ml}^{-1}$	$2 \times 10^6 \text{ ml}^{-1}$		
γ -irradiated BALB/c	Ultraviolet-irradiated C57BL/6J	+++	300
Ultraviolet-irradiated BALB/c	γ -irradiated C57BL/6J	—	≤ 1
Ultraviolet-irradiated BALB/c	γ -irradiated CBA/H	++	50
Ultraviolet-irradiated C57BL/6J	γ -irradiated CBA/H	+	10

* Stimulating spleen cell populations were prepared by mixing equal volumes of each component at the densities shown. *In vitro* cultures were then prepared by mixing 5 ml of the stimulating cell population with 5 ml of C57BL/6J lymph node cells at a density of $4 \times 10^6 \text{ ml}^{-1}$.

† Relative potencies were calculated from Fig. 2 by measuring the displacement along the abscissa of the different dose response relationships. The potency of the cultures stimulated with ultraviolet-irradiated BALB/c spleen cells was arbitrarily taken to be equal to or less than unity.

The displacement along the abscissa of the dose response relationship obtained from different cell populations is a measure of their relative potency.

These experimental findings were reproducible. γ -Irradiated BALB/c spleen cells produce a vigorous blast cell response when cultured with C57BL/6J lymph node cells, and generate a high titre of cytotoxic cells specific for the H-2^d antigen. Ultraviolet-irradiated BALB/c spleen cells, in contrast, produce no detectable cytotoxic response and no blast transformation. When γ -irradiated CBA/H spleen cells were added to cultures of ultraviolet irradiated BALB/c spleen cells and C57BL/6J lymph node cells, however, blast transformation was evident and the cytotoxic cell response to the H-2^d antigen was increased by five-fold over the CBA/H background.

The *in vitro* production of cytotoxic lymphocytes has been shown to be a function of activated T cells⁸. Thus, the above data indicate that transplantation antigens presented on the surface of the ultraviolet-irradiated lymphoid cells are poorly immunogenic for T cells *in vitro*. The *in vitro* immunogenicity of this antigen is significantly enhanced, however, if the responsive cells receive an allogeneic stimulus at the same time as

they are confronted with antigen. These findings confirm our earlier *in vivo* studies showing that allogeneic transplantation antigens are relatively weak in the absence of allogeneic stimulation^{1, 9}. They also raise the possibility that antigenic differences between donor and recipient may not constitute a major barrier to allotransplantation.

According to our hypothesis¹, lymphoid cells carried in the transplanted organ or tissue may effectively constitute the major transplantation barrier because of the way in which they influence the immunogenicity of transplantation antigen.

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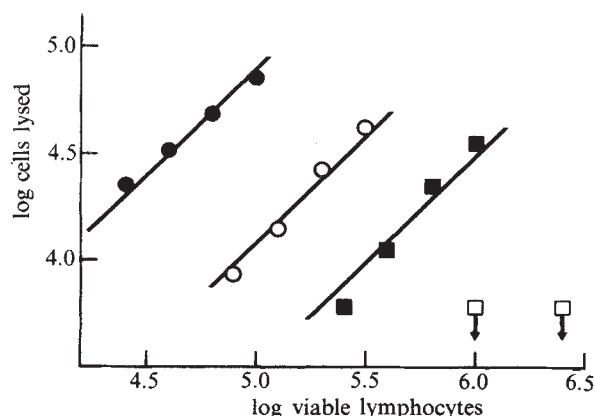


Fig. 2 Potency of cytotoxic cell populations generated *in vitro*. C57BL/6J lymph node cells were stimulated with the following spleen cell populations: ●, γ -irradiated BALB/c + ultraviolet-irradiated C57BL/6J; ○, ultraviolet-irradiated BALB/c + γ -irradiated C57BL/6J; ■, ultraviolet-irradiated C57BL/6J + γ -irradiated CBA/H; □, ultraviolet-irradiated C57BL/6J. Cell populations were assayed against 2×10^6 P815 mastocytoma target cells.

Cannabinoid content of some English reefers

This paper reports the results of qualitative and quantitative analysis of 36 reefers or 'pro-reefers' (samples of herbal or resin cannabis sufficient for one reefer) produced in London and Leeds. As far as we know it is the first time reefers in actual use have been so analysed. The reefers were obtained from three different groups: group A, regular smokers in the London area, some of whom had asked for psychiatric help; group B, regular users in Leeds, none of whom had sought medical advice about their cannabis smoking; group C, casual users in the London area. The results show a very wide variation in potency and indicate that more than half were below the threshold dose.

The contents of each reefer were weighed, examined macroscopically and microscopically and analysed¹ quantitatively for the main cannabinoid THC (Δ^1 -tetrahydrocannabinol), CBN (cannabinol) and CBD (cannabidiol) (Table 1). Almost all the reefers contained tobacco mixed with varying proportions of resin or herbal cannabis which, when possible, was separated and weighed.

We found a very high variation in potency, the content of the psychoactive substance THC varying from 0.15 mg to 41.1 mg.

As this variation has been found in only 36 samples, it is almost certain that similar or greater variation occurs regularly. In view of the known variability in the THC content of the plant (*Cannabis sativa* L.) from which the drug is obtained, the instability of the active constituents, especially in badly prepared and stored material and the 'unstandardised' conditions in which the drug is distributed the variation is perhaps not surprising. The lack of standardisation means that a casual smoker, used to low doses, may be accidentally exposed to highly potent material.

On average each reefer in groups A and C was smoked by two people (Table 2) so that the mean dose of THC per person in group A would be 4 mg (range 0.1 to 14 mg); and in group C would be 2.2 mg (0.5–5.0 mg). For group B, with an average of three people per reefer, the dose would be 3.5 mg (0.2–13 mg). These doses are lower than those used by such workers as Neumeyer and Shagoury² (2–9 mg) and Isbell *et al.*³, who quote 3.5 mg as a threshold dose and 16.2 mg sufficient to produce distinct depersonalisation. On this basis about two-thirds of the reefers, when shared, would produce effects less than that of the threshold value of 3.5 mg THC. Casual smokers may therefore be exposed to extremely small doses and so may falsely assume that cannabis is a relatively harmless substance. Conclusions based on questionnaires to smokers⁴,

Table 1 Components and analytical data on reefers

Reefer No. (apart from tobacco*)	Contents	Weight including tobacco (g)	Cannabinoids (mg)		
			THC	CBN	CBD
Group A					
1	Soft resin: (0.214 g)	0.744	28.39	traces	16.68
2	Soft resin	0.752	24.98	traces	14.65
3	†Leaf only: sessile glands (0.511 g)		11.13	traces	traces
4	†Coarse resin (0.118 g)		8.21	3.10	2.74
5†	Compact smooth resin (0.219 g)		5.99	2.73	6.60
6	Resin: fine powder	0.778	5.06	0.89	1.76
7	†Crumbly resin (0.224 g)		3.72	1.23	9.36
8	Greenish brown resin (0.102 g)	0.996	3.34	0.55	3.01
9	†Compact resins (0.327 g)		2.70	12.14	30.76
10	†Brown prism (0.278 g)		2.06	5.38	9.51
11	†Compressed herb (0.285 g)		1.97	0.36	2.85
12	†Leaf only: sessile glands (0.097 g)		1.89	traces	traces
13	Herbal: stalks and seeds	1.135	0.52	4.35	0.64
14	Herbal: tops and seeds	0.460	0.15	0.74	0.11
Group B					
15	Leaf only, numerous sessile glands	1.724	41.11	traces	traces
16	Herb: bracts, leaf, seeds	1.730	18.05	4.89	traces
17	Herb: stalk, leaf, seeds	1.309	17.56	4.50	traces
18	Leaf only: sessile glands	1.166	12.92	traces	traces
19	Herb: leaf mainly	1.096	11.85	traces	traces
20	Herb: stalks, leaf, seeds	0.698	9.56	1.89	—
21	Leaf: flowering tops, seeds	1.202	7.31	1.74	—
22	Herb: leaf with sessile glands	0.887	7.11	0.83	4.25
23	†Herb: seeds and leaf (0.317 g)		4.48	1.09	—
24	†Herb: bracts, seeds (0.440 g)		3.65	1.05	—
25	Resin: greenish brown	0.941	2.52	0.52	0.75
26	†Leaf and seeds (0.182 g)		1.38	0.16	0.35
27	Herb: bracts, seeds	0.842	0.38	0.16	0.32
28	†Fragments of green glass embedded in vegetable debris	0.208	0	0	0
Group C					
29	Resin: fine powder	1.046	9.37	0.99	5.99
30	Herbal: bracts and leaves	0.910	8.38	0.86	1.36
31	Resin: small lumps	0.553	5.59	0.67	3.64
32	Herbal: unripe floral axis, immature seeds	0.383	5.02	0.71	traces
33	Herbal: flowering tops, no seeds (0.224 g)		3.00	0.33	1.25
34	Herbal: with traces of resin	0.808	2.02	traces	traces
35	Resin: small lumps	0.637	1.38	0.36	2.82
36	Resin: greenish lumps	0.516	0.99	0.26	2.03

* All reefers contained tobacco except no. 15.

† Pro-reefers, consisting of resin or herbal cannabis sufficient for one reefer.

Table 2 Mean daily use of reefers

Reefer No.	Frequency of use (d ⁻¹)	No. of users	THC consumed (mg per person per d)
Group A			
1	7	1	199
2	7	1	175
3	5 to 10	1	56 to 111
6	20 to 30	2	51 to 76
7	30	1 to 2	4 to 11
8	10 to 30	2	17 to 50
9	9	1	24
13	20	2	5
14	20	2	1.5
Group B			
15	1	1	41
16	3	2	27
17	10	2 to 5	35 to 88
18	2.5	2	16
19	8	4	24
20	6	2	26
21	4	1 to 2	15 to 29
22	20	4	36
23	11	2	25
24	3	1	11
25	20 to 40	2	25 to 50
26	15 to 18	5 to 6	3 to 5
27	15 to 25	3 to 5	1 to 3
Group C			
29	4	3	19
30	1*	1	1
31	4	1	22
32	3	1	15
33	2	1	6
34	2*	1	0.6
35	2*	3	0.1
36	2*	3	0.1

* (per week)

such as students, are probably of little value unless adequate information on the potency of the reefer is also obtained.

All these doses refer to the actual amounts of THC in the reefers; obviously the amount reaching the blood stream will be affected by the manner in which the reefer is made and, more importantly, whether the user inhales and if he does how long he holds the smoke before expelling it⁵.

A more important variable is the actual number of reefers smoked by an individual per week. By careful questioning group A was found to consume 3.8 g cannabis per person per d (range 2 g to 6 g); group C 0.3 g (range 0.1 g to 1 g). For group B drug histories were collected using the techniques of participant observation⁶ and actual weighing of the amounts used. The average amount was 2.8 g per person per d (range 0.3 g to 8.3 g). As the reefers (Table 1) were collected at the time this information was obtained, it is possible to calculate the daily intake of THC on the assumption that the reefer analysed represented those being smoked at that time. The results (Table 2) show that for group A the average daily dose of THC is 60 mg per person, for group B 26 mg per person and for group C 8 mg. If the first two extremely high values of group A are removed the average for the remainder is 37 mg per person. Although these first two values are high they are not dissimilar from the values of 150 mg THC d⁻¹ quoted by Miras for regular users in Greece⁷. Some users evidently compensate for low potency reefers by smoking 10–20 reefers d⁻¹ and in these circumstances the reported carcinogenic effects^{8,9} may become significant.

Observation of some individuals, recorded before the analytical results were known, confirms a dose-response relationship. For reefer 15 (41 mg THC per dose) the user admitted he could not smoke more than 1 d⁻¹, otherwise he was unable to co-ordinate his movements. Reefer 22 (36 mg THC d) was from two females whose most characteristic feature was a persistent hilarity; neither was in full time employment. The suppliers of reefers 3 and 8 (17 to 111 mg) were all referred for mild cannabis psychoses, and were depressed and paranoid when smoking heavily. These symptoms disappeared within

2 months of giving up the drug. Reefer 27 (1–3 mg THC d) was from two small subgroups; none of their members exhibited noticeable disorganisation of the cognitive processes and all seemed entirely capable of holding full time employment. They seemed similar to the members of group C (casual smokers) who were in full time study or employment. It is significant that students reduced their intake or gave up the drug altogether before examinations. Subjects who used reefer 28 (glass fragments plus incense) made it from what they had bought as Turkish pollen hash at £18 per ounce. Although they obviously expected a good high they experienced no subjective euphoria; most reported serious headaches instead.

We found no evidence of adulteration except in reefer 28. This indicates that there is currently no shortage of genuine cannabis. Since THC decomposes into the inactive CBN due to faulty preparation and prolonged storage, the relatively high proportion of CBN in the group A reefers compared with group C indicates significant breakdown. This may come as a surprise to group A as they claimed to be nearer the sources of supply of cannabis, and therefore assumed they were using fairly fresh and active material.

Reefer 3 was grown out of doors in Brixton, London, and collected in July. Reefer 12 was grown in Dublin from Nigerian seeds and "harvested too early". Reefer 15 was from seeds taken from Zambian 'bush' and grown on a railway embankment near Leeds; the plants were harvested in September. None of the samples contained the relatively potent flowering tops, yet they had quite high THC content (21.8, 19.8 and 23.9 mg g⁻¹ air-dried leaf respectively). These results should therefore dispose of the idea that potent material cannot be grown in a cool climate on unobtrusive sites sometimes with poor lighting conditions. We hope to publish soon details of work on plants grown in this country over several years, which confirm this conclusion. We have already pointed out¹⁰ the defect in the present international definition of cannabis as "the flowering or fruiting tops of the cannabis plant (excluding the seeds and leaves when not accompanied by the tops)"¹¹.

Some of the reefers used by experienced smokers were low in THC content but nevertheless were claimed by the users to be satisfactory. One possible explanation is the higher average CBD figures for group A (7.0 mg) than for group C (2.1 mg). CBD is said to enhance the effect of THC¹². Furthermore, since this work began, evidence has accumulated that the GLC peak corresponding to CBD sometimes includes small amounts of other cannabinoids¹³. Synergism cannot be ruled out, and another possible explanation is the presence of noncannabinoid active material in cannabis.

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Evolutionary divergences between populations of Australian wild rabbits

THE first successful introduction of the European wild rabbit, *Oryctolagus cuniculus*, into Australia took place in 1859 when two dozen animals were imported into Geelong, Victoria¹. Subsequent minor introductions may have occurred but the evidence indicates that existing populations are derived mainly from the Geelong colony². Rabbit populations now occupy varied environments across the southern two-thirds of Australia³. These rabbits provide a good opportunity for the study of evolutionary divergences.

Twenty-six measurements were taken on the skulls of adult rabbits collected from five localities in Australia (Fig. 1). The numbers in each sample were: Tero Creek, 22; Manilla, 12; Tidbinbilla, 20; Holt Rock, 20; Wanneroo, 14. The environments of the localities are diverse. This diversity is reflected in the variations in mean annual rainfalls of the

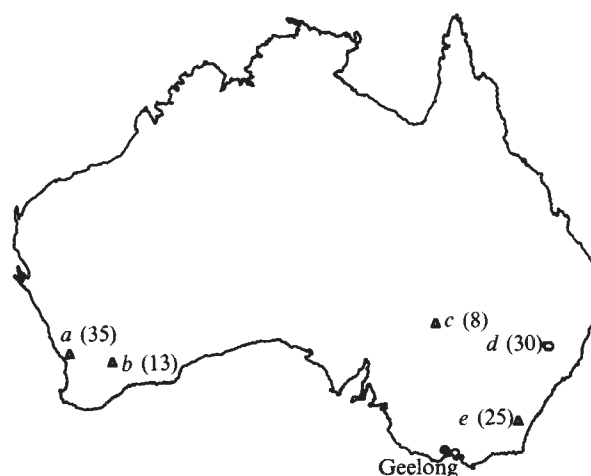


Fig. 1 Locations of collection sites. Δ Site of an extant collection; $\circ$, site of the early Manilla sample. The localities are, a, Wanneroo, WA; b, Holt Rock, WA; c, Tero Creek, NSW; d, Manilla, NSW; e, Tidbinbilla, ACT. The figures in parentheses indicate the approximate mean annual rainfalls (in inches) of the collection sites.

collection sites (Fig. 1). The Manilla sample was collected in 1918 (Australian Museum) and the Tero Creek sample in 1967 (CSIRO). The remainder were captured in 1972 and 1973 using ferrets, traps, spotlights and shooting. We regard all but the Manilla sample as extant.

The metrical parameters consisted largely of standard length, breadth and height measurements of the cranium, mandible and teeth. All parameters were tested for sexual dimorphism using *t* tests but only bizygomatic breadth showed significant sexual dimorphism. This measurement was eliminated from the remaining analyses. Univariate comparisons demonstrated a number of significant differences between all pairs of samples. The number of signifi-

cant *t* tests (0.05 level) varied from a low of 4 for the Wanneroo-Holt Rock comparison to 16 for the Wanneroo-Tero Creek and Wanneroo-Tidbinbilla comparisons. Over the 10 sample pair comparisons, the mean number of significant *t* tests was 10 out of 25. The parameters that showed significant differences varied considerably from one pair to the next so that most parameters showed significant heterogeneity in at least one sample pair comparison. Coefficients of variation for all characters in each of the regional samples were calculated. These coefficients generally were low⁴, with a mean of 3.8 and range of 1.2–9.6. A preliminary analysis of the same data using Penrose's⁵ distance statistics indicates a strong correlation between the geographic and biological distances between sample pairs. This finding is being investigated further.

The appearance of morphological heterogeneity between Australian rabbit populations in such a short time (110 yr) implies a fast rate of evolution. Skulls collected during the early stages of colonisation were not available, but some approximate evolutionary rates were calculated using the two most separated extant samples, Wanneroo and Tidbinbilla. The midpoint between the means of these two samples was taken as the ancestral value of each measurement. Our method requires the working assumption that the extant groups have diverged at the same rate from the hypothetical ancestral population.

Evolutionary rates for all measurements were calculated using Haldane's equation⁶, $\text{rate} = (\log_e X - \log_e Y)/t$ where $\log_e$ is the natural logarithm, *X* the mean value of descendants; *Y* the ancestral mean and *t* the elapsed time (set at 110 yr). Haldane suggests that the results be expressed in darwins, where one darwin is a change of 1/1,000th part in 1,000 yr. Over all parameters a mean rate of 116 darwins was obtained, with a range of 27–406 darwins. This mean rate of evolution is 5,000–10,000 times the estimated rate in teeth of horses over a period of 5–16 million years (ref. 6). The mean for the rabbit populations however is similar to the rates found for changes in the dental dimensions in a population of the African green monkey (*Cercopithecus aethiops sabaeus*) isolated for 300 yr on the West Indies island of St Kitts⁷. In this case the mean rate was 160 darwins with a range of 10–470 darwins⁷. These rapid rates of evolution would not be expected to continue indefinitely but to slow down as populations approached a genetic equilibrium.

Our results indicate that substantial morphological divergences have developed rapidly between Australian rabbit populations.

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Claim that two human linkage groups carry different loci for GPT and LDH withdrawn

CHEN and Giblett have reported the occurrence of genetic polymorphism for human red cell glutamic pyruvic transaminase (GPT; EC 2.6.1.2). The usual phenotypes of red cell GPT (that is, GPT 1, 2-1 and 2) could be identified on cellogel by adapting the starch gel electrophoretic method of Chen and Giblett to cellogel (Fraser *et al.*, unpublished). When a buffer system which separates the human and Chinese hamster forms is used, however, the isozyme patterns formed by fibroblasts were found to be distinctly different from those by red cells. We have employed the fibroblast isozymes as markers in human gene linkage studies using man–Chinese hamster somatic cell hybrids and have reported that the enzyme with GPT activity is probably a tetramer determined by two genes named *GPT B* and *GPT C* located on different chromosomes and linked to *LDH B* and *LDH A* respectively².

In a later series of experiments involving further characterisation of the GPT isozymes in the hybrids and their parental cells, we have found strong evidence that the screening procedures used do not score different enzymes (GPT and LDH) but the same gene product (LDH) by different methods.

The principle of staining for GPT is that the enzyme converts alanine and α -ketoglutarate to glutamic acid and pyruvate, and the pyruvate thus formed is detected by coupling it to LDH when NADH is oxidised to NAD⁺. The sites of enzyme activity are seen under long wave ultra-violet light as quenched areas against a fluorescent background. If the appearance of bands was caused by the action of GPT isozymes, an omission of any of the substrates in the reaction mixture should prevent the formation of bands.

It turned out that an omission of alanine alone or alanine and LDH in the reaction mixture did not prevent the appearance of any of the bands seen after staining with the complete GPT reaction mixture. Moreover, a substitution of α -ketoglutarate by pyruvate in the staining mixture resulted in the same electrophoretic pattern. With pyruvate as substrate the bands appeared instantaneously with increased intensity, whereas with α -ketoglutarate the gels had to be incubated at 37°C for at least 30 min for the patterns to become visible.

These observations show that the human and Chinese hamster lactate dehydrogenase isoenzymes are capable of utilising α -ketoglutarate as a substrate analogue in the conditions used and that these conditions are obviously not optimal for the GPT of the species concerned. Therefore, the patterns observed earlier by us² on staining for glutamic pyruvic transaminase in the cultured fibroblasts, white blood cells and the hybrids are probably due to lactate dehydrogenase activity, indicating that the GPT-LDH linkages were spurious.

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Connective stability of complex ecosystems

IN 1970, Garner and Ashby¹ asked an important question: "If a large system is assembled (connected) at random, or has grown haphazardly, should we expect it to be stable or unstable?" They showed on a linear model that probability of stability generally decreases with the increase of the degree of connectedness among the parts of the system. This fact was confirmed by May² in a study of large systems in the ecological context.

Here I now report that a wide class of linear and non-linear time-varying large systems have been identified^{3,4}, which are stable in spite of changes in the degree of connectedness among the parts (subsystems) of the system. A large system is considered connectively stable if it is stable (in the sense of Liapunov) for any degree of connectedness among the subsystems. The connective property of stability can be established in the systems with competitive equilibrium⁵, which appear as useful models in such diverse fields as biology⁶ and the arms race⁷, economics⁸ and transistor circuits⁹.

The development of the connective stability concept can be best illustrated on the linear model used in (refs 1 and 2):

$$\frac{dx}{dt} = Ax$$

where $x = \langle x_1 \ x_2 \ \dots \ x_n \rangle$ is an n vector and $A = (a_{ij})$ is an $n \times n$ constant matrix. I assume that A has negative diagonal elements¹, and non-negative off-diagonal elements, that is

$$a_{ij} \begin{cases} < 0, & i=j \\ \geq 0, & i \neq j \end{cases}$$

The above system is stable if (and only if) the matrix A satisfies the Hicks conditions:

$$a_{11} < 0, \quad \begin{vmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{vmatrix} > 0, \dots, (-1)^n \begin{vmatrix} a_{11} & a_{12} & \dots & a_{1n} \\ a_{21} & a_{22} & \dots & a_{2n} \\ \dots & \dots & \dots & \dots \\ a_{n1} & a_{n2} & \dots & a_{nn} \end{vmatrix} > 0$$

I allow off-diagonal elements a_{ij} to be time-varying, and replace them with $e_{ij}a_{ij}$, where $e_{ij} = e_{ij}(t)$ are arbitrary functions such that

$$0 \leq e_{ij}(t) \leq 1$$

Therefore, the elements $e_{ij}(t)$ of the $n \times n$ interconnection matrix $E = (e_{ij})$ measure the coupling from x_i to x_j and represent disconnection when $e_{ij}(t) = 0$ at any time t . It has been shown^{4,8} that, under Hicks conditions, stability of the system is invariant to changes in any (and all) $e_{ij}(t)$ and that the system is connectively stable.

In an ecological system, x is the vector of the populations x_i and the elements a_{ij} of the matrix A represent the effect of the j th species on the i th species in the neighbourhood of the equilibrium. Non-negativity of the off-diagonal elements $a_{ij}(i \neq j)$ implies (gross) symbiotic interactions among species. Negativity of the diagonal a_{ii} s means that each species is density dependent or 'stabilized'². Both restrictions on the sign of a_{ij} s can be removed at the expense of more refined analysis. By applying the Hicks conditions to the McKenzie's diagonal form of A , the off-diagonal a_{ij} s can have arbitrary signs and connective stability of the ecosystem with mixed (competitive - predator - symbiotic - saprophytic) interactions among species can still be established. The restriction $a_{ii} < 0$ can be removed by considering species in the stable 'blocks' (subsystems) and using the decomposition-aggregation methods^{3,4,8} to conclude stability of the overall system by Hicks conditions. The decomposition-aggregation analysis can take advantage of special structural 'block' properties of ecosystems². Furthermore, it is possible to demonstrate⁸ wide tolerances to nonlinearities in the interactions of stable

ecosystems reflected by non-linear elements $a_{ij} = a_{ij}(t, x)$ of the system matrix $A = A(t, x)$.

By increasing the magnitude of the off-diagonal elements a_{ij} and thus the interaction complexity of the ecosystem, the Hicks conditions are eventually violated. Since the conditions are both necessary and sufficient for stability, by reaching the critical values of a_{ij} s as determined by the Hicks inequalities, a sharp transition from stability to instability takes place. Therefore, there is an analytical basis^{3,4,8} for verification of the important Garner-Ashby conjecture¹ and the equally significant May's experiment².

The recursive nature of the Hicks conditions suggests that individual parts of the system can be decomposed from each other and again composed together randomly in various ways without affecting stability. It should be noted, however, that Hicks conditions are valid only for the constant matrix A . Kamke's comparison principle and vector Liapunov functions from the theory of differential inequalities¹⁰ are needed to extend the validity of Hicks conditions to matrices with time varying elements and to give a rigorous proof^{4,8} of this intuitive result.

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Palatability dynamics of cardenolides in the monarch butterfly

THE adaptive strategy of sequestering cardiac glycosides from milkweed plants (Asclepiadaceae and Apocynaceae) has evolved in several taxa of insects¹. Since these cardenolides elicit vomiting following ingestion, birds learn to avoid the insects on sight after one or more emetic experiences². By a specific assay, we found a spectrum of cardenolide concentrations in adult monarch butterflies (*Danaus plexippus* L., Danainae) collected during the autumnal migration from four areas in eastern North America³. In this communication we compare Atlantic with Pacific Coast monarch populations and explore quantitative relationships between cardenolide concentrations and palatability spectra. The latter were measured by our blue jay (*Cyanocitta cristata* *bromia* Oberholser, Corvidae) emetic dose fifty (ED₅₀) test⁴.

Migrating East Coast butterflies were netted in September 1971 in the floodplain of the Connecticut River in Northampton and Hockanum, Massachusetts. Those from the West Coast came from three overwintering colonies in California during November 1971 at Muir Beach, Natural Bridges State Park at Santa Cruz, and at the Academy of Music at Santa Barbara. All butterflies were frozen until oven dried and individually ground for analysis as before³. Cardenolide concentrations were measured from February to June 1972. Because there were no significant concentration differences between the California populations, they were combined for statistical analyses.

The spectrophotometric assay³ was modified as follows: (a) a correction in the alignment of the visible light source of the Perkin-Elmer 402 spectrophotometer was made; (b) absorbances were read at the peak (626 nm); (c) a tendency for opalescence⁵ was eliminated by changing the aqueous NaOH solution to 50% EtOH : 50% H₂O. These modifications resulted, respectively, in corrections of 1.08, 1.18 and 1.11, to give a combined correction factor of 1.42. As previously, the *F* peak (536 nm) of a holmium oxide filter was used to monitor machine performance ($n=83$; $\bar{X}$ absorbance = 0.377; s.d. = 0.003).

ED₅₀ tests were done between March 1972 and February 1973 on blue jays trapped in Hampshire County, Massachusetts between December 1971 and February 1973. They were stored for various times in an outside aviary or inside in a 12 h light : 12 h dark regime. Birds given Massachusetts butterfly material were force fed once. Thirty-three birds given the California 0.250–0.279 and 0.320–0.399 subsample material (Table 2) had been force fed once, at least 5 weeks previously. The mean weight of the 226 birds was 84.25 g ($s^2=41.83$).

After determining cardenolide concentrations in 351 California and 355 Massachusetts butterflies (Table 1, Fig. 1), we combined the residual dry powders of both sexes into nine concentration range subsamples for California and eleven subsamples for Massachusetts (Table 2); as all residual powders were used, larger butterflies contributed more than smaller ones. After mixing, we measured the absorbance per 0.1 g of each subsample.

We then determined the ED₅₀ values for most subsamples (Table 2). The lower concentration groups for both coasts either were not emetic or were subemetic so that these ED₅₀ values could not be determined. The ED₅₀ and subsample absorbance values were run in regression analyses by the method of least squares⁷ to fit the exponential equation $y = ax^c$, giving high correlation coefficients (Table 3D). Because of the exponential relationship in both samples, the mean values and 95% confidence limits are plotted logarithmically together with regression lines (Fig. 2).

Concentrations are summarised in Tables 1 and 2 and in Fig. 1, which also shows the distribution of total cardiac glycoside per butterfly, obtained as the product of individual absorbance $\times$ dry weight. The histograms for the Massachusetts butterflies confirm the earlier study³ and are essentially normal distributions showing continuous variation in cardenolide content. More than 98% of the Massachusetts monarchs contained substantial amounts of cardenolide. The population of California monarchs, however, was effectively dimorphic, with 47% of the individuals containing little to immeasurable amounts.

As Fig. 1 and Table 1A show, California butterflies had a much lower mean cardenolide content than the Massachusetts

butterflies, even when values were recalculated after excluding all butterflies with absorbances of less than 0.100 per 0.1 g (Table 1B). With this group excluded, the variance of the cardenolide content in both sexes was three to four times greater in the Massachusetts butterflies. In both samples females had higher concentrations than males. The two-way analyses of variance⁸ indicate that both geographic and sexual differences in concentration were highly significant.

The size (right forewing length, measured with calipers from base to apex) and dry weight of the butterflies were highly

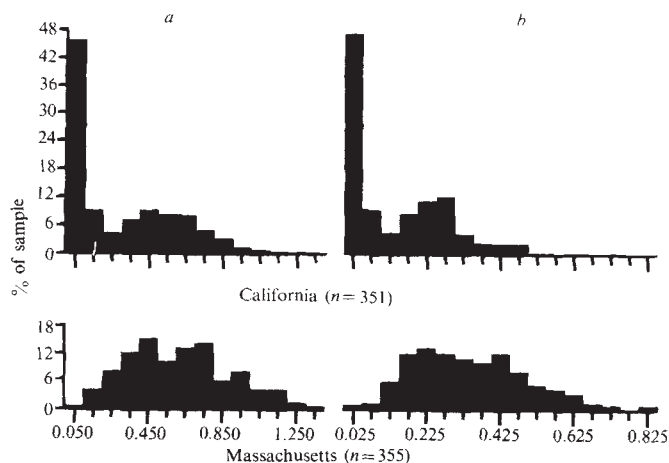


Fig. 1 Variation in cardiac glycoside content of monarch butterflies from California and Massachusetts (data are in Table 1). a, Total cardiac glycoside (absorbance per butterfly); b, cardiac glycoside concentration (absorbance per 0.1 g butterfly). The West Coast monarchs are bimodal, with nearly half containing little to immeasurable amounts of cardiac glycosides. The East Coast monarchs are unimodal and have a substantially higher mean cardenolide content. An extract of 0.1 g of butterfly in 5 ml of alcohol which gives an absorbance of 0.200 by our standard method is equivalent to a 3.16×10^{-5} M solution of digitoxin, or 241 μ g per 0.2 g butterfly.

correlated (Table 3B)⁹. The males were heavier as well as larger than the females on both coasts (Table 1C and D). California butterflies, however, although significantly smaller than their Massachusetts counterparts, were substantially heavier, no doubt because of a higher fat content (observed during grinding). Moreover, California butterflies showed greater discrepancy between the sexes for both weight and size (interactions). The differences in cardenolide content between the sexes, exaggerated in the California butterflies (Table 1A and B), support the idea³ that there is a significant physiological

Table 1 Summary of cardenolide concentrations (absorbance per 0.1 g powdered butterfly), dry weights and right forewing lengths of Massachusetts and California butterflies

(A) Cardenolide concentration: all butterflies							Two-way analysis of variance					
Massachusetts			California			Variation	ss	df	ms	F	P	
Mean	Variance	n	Mean	Variance	n							
Males	0.317	0.018	186	0.115	0.012	190	Sex	0.135	1	0.135	7.314	<0.01
Females	0.349	0.025	169	0.139	0.020	161	Coast	7.472	1	7.472	404.061	<0.001
Totals	0.332	0.021	355	0.126	0.016	351	Interact.	0.002	1	0.002	0.158	>0.25
(Ratio of Mass.: Calif. variances = 1.313, $P < 0.05$)							Residual	12.983	702	0.018		
(B) Cardenolide concentration: absorbances ≥ 0.100							Two-way analysis of variance					
Mean	Variance	n	Mean	Variance	n	Variation	ss	df	ms	F	P	
Mean	Variance	n	Mean	Variance	n							
Males	0.323	0.017	182	0.228	0.004	84	Sex	0.192	1	0.192	12.163	<0.001
Females	0.354	0.024	166	0.281	0.008	71	Coast	0.753	1	0.753	47.600	<0.001
Totals	0.338	0.020	348	0.252	0.006	155	Interact.	0.013	1	0.013	0.827	>0.50
(Ratio of Mass.: Calif. variances = 3.33, $P < 0.001$)							Residual	7.903	499	0.015		
(C) Dry weight (g)							Two-way analysis of variance					
Mean	Variance	n	Mean	Variance	n	Variation	ss	df	ms	F	P	
Mean	Variance	n	Mean	Variance	n							
Males	0.191	0.0007	186	0.234	0.0017	190	Sex	0.028	1	0.028	20.156	<0.001
Females	0.185	0.0007	169	0.215	0.0024	161	Coast	0.235	1	0.235	169.505	<0.001
Totals	0.188	0.0007	355	0.225	0.0020	351	Interact.	0.005	1	0.005	4.081	<0.05
(Ratio of Mass.: Calif. variances = 1.313, $P < 0.05$)							Residual	0.977	702	0.001		
(D) Length of right forewing (cm)							Two-way analysis of variance					
Mean	Variance	n	Mean	Variance	n	Variation	ss	df	ms	F	P	
Mean	Variance	n	Mean	Variance	n							
Males	5.17	0.041	186	5.13	0.046	190	Sex	1.008	1	1.008	21.037	<0.001
Females	5.15	0.038	169	5.00	0.068	161	Coast	1.500	1	1.500	31.296	<0.001
Totals	5.16	0.040	355	5.07	0.056	351	Interact.	0.570	1	0.570	11.908	<0.001
(Ratio of Mass.: Calif. variances = 1.313, $P < 0.05$)							Residual	33.652	702	0.047		

Table 2 Subsample determinations of cardenolide concentrations and emetic dosages for California and Massachusetts butterflies

	Cardiac glycoside content of butterflies (absorbance per 0.1 g dry weight)				Absorbance of combined subsample	No. of birds tested	Emetic dosage of butterflies (g of dry butterfly per 100 g of bird)		
	No. of butter- flies in subsample		% of total sample	Absorbance range of subsample			ED ₅₀	95% Confidence limits of ED ₅₀	Estimated no. of ED ₅₀ units*
C									
a	66†	56†	35	0.000 to 0.024	0.014	9†	Indeterminate		
l	24	20	12	0.025 to 0.049	0.030	9‡	Indeterminate		
i	26	18	12	0.050 to 0.149	0.100	11§	Indeterminate		
f	19	8	8	0.150 to 0.199	0.171	15	0.123	0.098 to 0.153	2.8
o	22¶	15¶	11	0.200 to 0.249	0.228	14	0.051	0.046 to 0.057	4.1
r	12	16	8	0.250 to 0.279	0.250	21	0.056	0.051 to 0.062	4.6
n	16	8	7	0.280 to 0.319	0.290	15	0.047	(0.045 to 0.049)¶	5.6
i	4	9	4	0.320 to 0.399¶	0.305	14	0.035	0.031 to 0.040	6.0
a	1	11	3	0.400 to 0.475**	0.420	12	0.037	0.035 to 0.039	9.1
Totals	190	161				120	(maximum estimate = 10.6)		
	351								
M									
a	4	3	2	0.000 to 0.099	0.085	2††	Indeterminate		
s	19	13	9	0.100 to 0.157	0.150	7‡‡	Indeterminate		
s	14	17	9	0.158 to 0.199	0.195	8§§	Indeterminate		
a	27	20	13	0.200 to 0.249	0.235	10§§	Indeterminate		
c	24	20	12	0.250 to 0.299	0.280	12	0.259	0.218 to 0.308	0.9
h	22	17	11	0.300 to 0.349	0.320	10	0.219	0.201 to 0.238	1.0
u	20	15	10	0.350 to 0.399	0.385	13	0.186	0.160 to 0.216	1.2
s	26	17	12	0.400 to 0.449	0.440	14	0.171	0.158 to 0.184	1.3
e	14	15	8	0.450 to 0.499	0.490	12	0.147	0.128 to 0.169	1.4
t	12	19	9	0.500 to 0.599	0.510	11	0.138	(0.131 to 0.145)¶	1.4
t	4	13	5	0.600 to 0.815**	0.635	7	0.150	0.133 to 0.169	1.7
s									
Totals	169	186				106	(maximum estimate = 2.0)		
	355								

* Based on regression lines in Fig. 2, the mean weight of the Californian or Massachusetts butterflies, and the mean weight of the blue jays.

† Combined sample included random selection of twenty-two males and seventeen females.

‡ No birds vomited; three birds dosed at each of three levels: 0.129, 0.192, and 0.287 g.

§ One bird each dosed at levels 0.086, 0.105, 0.129, 0.142, 0.173, 0.212, 0.287, 0.350, and 0.522 g. did not vomit; one bird each at levels 0.129 and 0.287 g vomited.

¶ Two males and five females in this range were not combined.

⦿ Estimate subject to error because $NB-A^2/N^2 = 0.250$ for California and 0.160 for Massachusetts.

|| One male of 0.013 absorbance was mistakenly included in the combined sample.

** The maximum concentration.

†† No birds vomited; dosed at 0.287 and 0.350 g.

‡‡ One bird dosed at 0.287 g vomited; two each dosed at 0.287, 0.350 and 0.428 g did not.

§§ Two birds dosed at 0.287 g did not vomit; at 0.350 g two did and one did not; at 0.428 g one did and two did not.

¶¶ Three birds dosed at 0.368 g did not vomit; at 0.407 g, three did and two did not; one bird each at 0.449 and 0.496 g did not vomit.

cost to sequestering these compounds.

The relationships of cardenolide concentrations and ED₅₀ values are shown in Fig. 2 and Table 2. Although there seems to be an upper limit for both the California and Massachusetts butterflies, generally the higher the concentration of cardenolide, the lower the dose needed for emesis. However, at all emetic concentrations, the California monarchs were more emetic than their Massachusetts counterparts. Within the range of overlapping absorbances, at 0.250 and 0.475, the Massachusetts ED₅₀ values were, respectively, 0.264 g and 0.162 g, compared with California ED₅₀ values, respectively, of 0.057 g and 0.025

g. In other words, within this absorbance range the California monarchs were at least 4.6 to 6.5 times as emetic. This no doubt mirrors the separate *Asclepias* floras on the East and West coasts¹⁰, several species of which are known larval food plants of the monarch¹¹.

Both dry weight and size were negatively correlated with cardiac glycoside concentration in the Massachusetts females, and significance was approached for the Massachusetts males (Table 3A and C). In contrast, neither sex in California showed these correlations. In order for the Massachusetts butterflies to be sufficiently emetic, they must incorporate

Table 3 Summary of linear regression analyses of cardenolide concentration (absorbance per 0.1 g powdered butterfly), dry weight (g), and right forewing length (cm)

		No. of data points	<i>a</i>	<i>c</i>	Correlation coefficient	Standard error	<i>P</i>
(A) Dry weight compared with concentration							
Massachusetts	males	186	-0.568	+0.427	-0.118	0.132	0.20 > <i>P</i> > 0.10
Massachusetts	females	169	-0.997	+0.534	-0.167	0.155	0.05 > <i>P</i> > 0.02
California	males	190	-0.316	+0.190	-0.119	0.109	0.20 > <i>P</i> > 0.10
California	females	161	-0.027	+0.145	-0.009	0.142	0.50 > <i>P</i> > 0.40
(B) Dry weight compared with right forewing length							
Massachusetts	males	186	+5.120	+4.187	+0.694	0.146	< 0.001
Massachusetts	females	169	+5.792	+4.079	+0.785	0.120	< 0.001
California	males	190	+2.568	+4.532	+0.498	0.186	< 0.001
California	females	161	+2.863	+4.381	+0.544	0.219	< 0.001
(C) Right forewing length compared with concentration							
Massachusetts	males	186	-0.087	+0.766	-0.132	0.132	0.10 > <i>P</i> > 0.05
Massachusetts	females	169	-0.192	+1.341	-0.237	0.153	<i>P</i> < 0.001
California	males	190	+0.043	-0.104	+0.084	0.110	0.40 > <i>P</i> > 0.20
California	females	161	+0.053	-0.126	+0.098	0.141	0.40 > <i>P</i> > 0.20
(D) ED₅₀ compared with absorbance per 0.1 g							
Massachusetts		7	+0.092	-0.757	-0.931	—	< 0.01
California		6	+0.009	-1.313	-0.875	—	< 0.025

For A, B and C, $y = ax + c$; for D, $y = ax^c$.

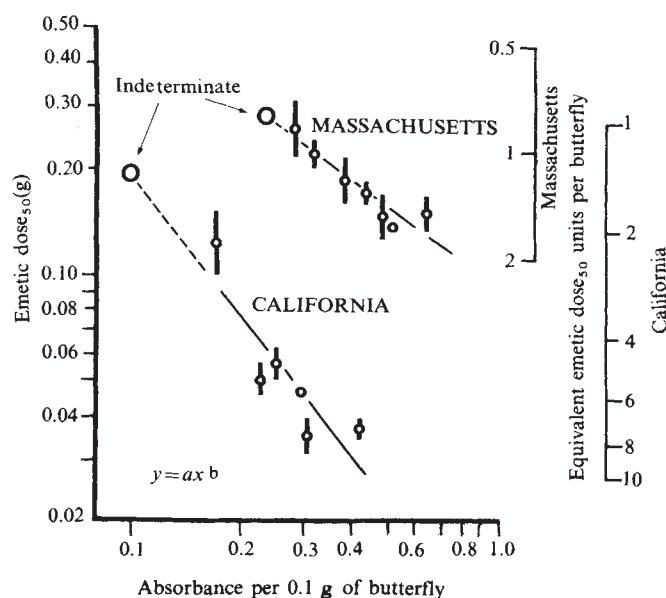


Fig. 2 Relationship of cardiac glycoside concentrations and ED_{50} values (data are in Table 2). The lines extending from the small open circles are the 95% confidence limits of the means; the dotted lines represent indeterminate to nonemetic concentrations. Emetic dose is inversely related to cardiac glycoside concentrations, thereby proving that the palatability spectrum in these wild monarch butterflies is causally related to a spectrum in cardenolide concentration. Cardenolides in the California butterflies, however, are far more emetic than those in the Massachusetts butterflies, no doubt reflecting the completely distinct *Asclepias* species on the two coasts from which the wild larvae sequester the poisons. The ranges in equivalent emetic dose 50 U per blue jay predator per butterfly are shown on the right side of the graph.

more cardiac glycoside than the California butterflies and these higher concentrations may be physiologically detrimental.

In terms of blue jay ED_{50} units, the Massachusetts butterflies ranged from an estimated maximum of 2.0 to less than 0.9, whereas the more toxic California butterflies ranged from 10.6 to 2.8. We did not resolve the number of ED_{50} units below these minima. There is, however, a concentration threshold below which the butterflies are effectively not emetic in terms of the numbers capable of being ingested per bird per unit time. For the California sample, this threshold is certainly not less than an absorbance of 0.050, so that at least 47% of the population is effectively palatable. A small percentage of the population (perhaps 5–10%) contains less than one emetic unit and is therefore of intermediate palatability, while the remaining 43–48% is unsuitable as food. For the Massachusetts sample, the threshold is not as clear-cut, but is probably near an absorbance of 0.150. Thus only about 10% of the Massachusetts population is effectively palatable, with a larger group of intermediate palatability (about 35%), leaving approximately 55% of the population with one to two ED_{50} units.

The mean absorbance per butterfly in Massachusetts reported³ in 1970 (0.198), when corrected (0.281), was 15% lower than found in 1971, ($t=3.42$, d.f.=475, $P<0.001$). However, the corrected absorbance values of one ED_{50} unit for the 2 yr are very similar: 0.327 for 1970 and 0.320 for 1971. In other words, the basic emetic properties per unit of absorbance remained virtually the same, but the mean concentration changed, probably because of a difference in environmental factors affecting cardiac glycoside production in the wild milkweeds during the 2 yr.

The fact that California and Massachusetts populations contain both palatable and unpalatable butterflies verifies the heretofore largely theoretical category of mimicry known as automimicry^{12,13}. In this type of mimicry, which is mathematically analogous to a perfect Batesian mimicry system, the palatable individuals of the species derive mimetic protection

from the unpalatable portion of the same population from which they cannot be visually distinguished. Automimicry theory predicts that the more noxious the unpalatable individuals are, the higher can be the proportion of palatables in the population. The greater percentage of completely palatable butterflies in the much more noxious California population compared with the Massachusetts population verifies this prediction. Moreover, the high degree of protection that must result from the very emetic California butterflies probably made possible the evolution of the spectacular overwintering clusters which are found on the California coast but not in the East.

The assumption that wild *Danaine* butterflies are uniformly unpalatable has played a key roll in the development of mimicry theory. This assumption can no longer be made, however, either in attempts to interpret older experiments¹⁴ or in future work. Our study makes it clear that unpalatability is a dynamic situation. We must also question whether all *Danaine* species are equally adept at sequestering cardenolides, as well as whether they are equally able to eat the various poisonous to nonpoisonous milkweed species available to them. Because of the dynamics of palatability, certain of the inconsistencies in the mimicry literature, such as the colour polymorphism found in some populations of supposedly unpalatable butterfly species¹⁵, now seem amenable to analysis. An interesting case is the widespread African *Danaus chrysippus* L. which is polymorphic in some parts of its range and monomorphic in others¹⁶. Where monomorphic, a substantial portion of the population is probably unpalatable. But in other areas, *D. chrysippus* may be effectively palatable and a polymorphic Batesian mimic.

The distinct differences in emetic properties of the cardenolides found in the Massachusetts and California butterflies suggests that more *Asclepias* species might profitably be investigated to discover new cardenolides which retain their useful attributes as heart drugs, but which have less noxious emetic side effects^{17,18}.

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Genetic selection of winner and loser rats in a competitive situation

GENETIC selection of behavioural characteristics has been observed, among others, in the Maudsley, Roman, and Tryon strain of rats¹. Here we describe an experiment designed to test the hypothesis that rats could be selectively bred to win or lose in a food competition situation of the type reported by Lindzey *et al.*² with mice and later used by other authors with rats³⁻⁸.

The apparatus is described in detail elsewhere⁵. Briefly, it consisted of a straight wood runway 100 cm long, connected at the extremities with two identical open-topped chambers; a guillotine door bisected the runway. The animals had food withheld for 20-22 h and were trained individually every 48 h to traverse the runway to be rewarded with food. They were then subjected to the contest situation, in which two rats were simultaneously introduced into the opposite sides of the apparatus. As the rats met in the middle of the runway, one pushed the other and gained the reward.

The offspring of three randomly selected couples of Wistar rats from our own colony, maintained by random breeding, were subjected to the competitive situation when about 80 d old. A stable hierarchy of winners and losers was obtained among seven females and seven males. The two male rats which consistently won were bred with the two females that were winners from their hierarchy. Similarly, the two loser rats of the female and male hierarchy were also bred. The offspring (F1) consisted of 15 rats which were descendants of winner couples (DW) and 14 rats which were descendants of loser couples (DL). At 80 d of age, competitive hierarchies were established independently for DW males, DW females, DL males and DL females. The two DW male rats which occupied the top of the hierarchy were mated with the two DW female rats which also occupied the first and the second positions. Similarly, the two DL males at the bottom of their hierarchy were mated with the two DL females which occupied the last two positions. Only one female and one male were put together for mating.

F2, consisting of 15 DL and 11 DW were tested against random-bred controls, using a fixed pair procedure. Each pair was made up by a DW or DL rat and a random-bred control of the same sex and age. Each pair was subjected to three experimental sessions consisting of five contests each. After that, the rats were selected for mating according to their number of wins (DW that won at least 13 out of the 15 contests and DL that lost at least 13 out of the 15 contests). F3 (31 DW and 31 DL) and F4 (32 DW and 32 DL), were

subjected to the same procedure as F2. In the three generations (F2, F3 and F4) tested against randombred controls, the DW rats won about 75% of the contests while the DL rats lost nearly 80% of the contests. Throughout the generations, mating brothers and sisters was avoided when possible. At the F1 and F2 about 50% of the couples which were mated were brother and sisters while this dropped to about 10% in the following generations.

In F5, 60 pairs were formed, each consisting of a DW and a DL rat of similar weight. Thirty-seven pairs were females and the remaining 23 males. Each pair, deprived of food for about 22 h, was tested using the fixed pair procedure. Three experimental sessions were run, consisting of five contests each. The results (Fig. 1) show that DW rats consistently won more than 80% of the 15 contests. In all generations, records of weights were kept and correlations between weight and number of wins were calculated. No significant correlations were found.

These data indicate that genetic selection occurred. DW and DL rats probably have different behavioural or physiological characteristics, which are yet to be detected, that led them either to win or lose in the straight runway. The possibility that the results obtained were not due to genetic selection but to an influence of the mothers during weaning was excluded, as in the F4 and in the F5 generation, all rats were reared by foster mothers.

How winning and losing in the straight runway correlates with other competitive situations remains to be investigated.

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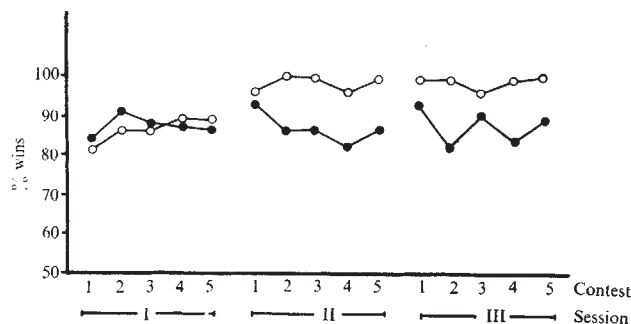


Fig. 1 Percentage of wins obtained by 23 DW males (●) and 37 DW females (○) when competing in a straight runway against 23 DL males and 37 DL females, respectively. The difference between the number of victories obtained and the casual level of 50% was significant in every contest. The statistical analysis was performed for females using the binomial test for large samples ($P \leq 0.001$) and using the binomial test for small samples for males ($P \leq 0.002$).

Infectious particles in a marine ciliate

INFECTIOUS, self-reproducing particles of unknown origin have been demonstrated in various tissues and cells^{1,2}, notably fresh water protozoa³, but not in marine protozoa. Advances in the development of culture media for marine ciliates⁴ and the need for simple model systems for biochemical study of intracytoplasmic particles have led us to survey marine protozoa. Of 104 isolates made from twenty-one seawater samples taken from tidal pools and brackish backwaters along the South Florida coast, twenty-six were cultivated axenically as described before⁴. Of these, four contained in their cytoplasm infectious, self-reproducing particles, which we have termed 'xenosomes' (*xenos*, alien; *soma*, body).

The xenosomes were found in aceto-orcein-stained preparations⁴ under the phase microscope (Fig. 1a). They seemed to be distributed randomly throughout the cytoplasm. We

have maintained one xenosome-bearing stock, a small hy-menostome ciliate identified as *Parauronema acutum* 110-3, for more than 2 yr, subcultured weekly, without loss of particles. The ciliates were maintained axenically in M medium⁴ modified to contain 500 $\mu\text{g ml}^{-1}$ asolectin as the sole source of lipid, at 22°C in the dark. From 10 l of late logarithmic phase culture containing approximately 10^6 animals ml^{-1} , we recovered as many as 8.0×10^{11} highly purified particles per attempt (Fig. 1d). The method of isolation and purification of the particles was essentially the same as that used with the mu and pi symbionts of *Paramecium aurelia*⁵. Xenosomes appear as rod-shaped particles, approximately 0.3 μm wide and 1.0 μm long. They occur exclusively in the cytoplasm in numbers reaching 300 per protozoan. When released from the protozoa (ruptured by gentle pressure of a glass coverslip) the particles can be seen spinning (propeller-like) and darting rapidly, suggesting the presence of a motile apparatus, perhaps flagella. Xenosomes are Gram negative and appear to divide within the cytoplasm by transverse fission, as evidenced by a furrow, seen in some particles, in a plane perpendicular to their long axis. The appearance of the particles in the electron microscope (*in situ*) suggests that they are bounded by a double-track membrane (Fig. 1c). Dispersed throughout the internal contents of the particle is a network of fibrous strands, which disappears after treatment with

TABLE 1 Comparison of infectivity of whole culture blendates (cell-free) with filtrates of xenosome-bearing *P. acutum* 110-3

Sample tested	As is	Sample dilution			
		1:2	1:4	1:8	1:16
Whole culture, blended and filtered	76	41	18	0	1
Filtrate only, no blending	80	39	17	3	0
Filtrate only, blended	75	52	28	7	0

Figures represent percentage of animals infected.

Cultures of xenosome-bearing *P. acutum* 110-3 in the exponential phase of growth (population density, $0.5 \times 10^6 - 1.0 \times 10^6$ animals ml^{-1}) were either blended in a sterile Waring blender to more than 95% cell breakage and filtered through a sterile Millipore filter (8 μm pores) to remove unbroken cells, or filtered directly without blending. Samples of 0.1 ml of the filtrates, or dilutions thereof, were added to 0.9 ml each of fresh modified M culture medium contained in the wells of nine-spot depression slides, inoculated with about 200 particle-free animals, and incubated at 22°C in the dark in humidifying chambers for 7–10d. About 5 μl of the cell suspension from each depression was then placed on a glass slide, exposed to osmium vapours (2% (w/v) OsO_4 in distilled water) for about 5 s and allowed to dry at room temperature. A small drop of aceto-orcein dye was added, followed by a coverslip, and the animals were viewed under oil immersion using phase optics in the Leitz microscope. Fifty animals from each depression were observed and scored for the presence of particles. The number of animals containing particles from triplicate depression slide cultures were averaged and the data expressed as percentage of animals infected. A filtered sample of the culture, blended for the same time as the whole culture, was included as a control.

DNase and, presumably, consists of DNA. Attempts to culture the particles outside the host in media similar to that used for the ciliates themselves have not been successful. Nor has it been possible to grow particles in media supplemented with biological materials and growth factors.

Purified particle preparations were fractionated by the Schneider procedure⁶ and the nucleic acids were extracted from the dry, de-fatted residues with hot trichloroacetic acid (TCA). The extract was analysed for DNA by the diphenylamine procedure⁷ or by the method of Webb and Levy⁸ and for RNA by the orcinol procedure⁹. Protein was determined by solubilising the TCA-extracted residue in 0.1 N NaOH and assaying the extract by the Lowry procedure¹⁰. The DNA, RNA and protein content of the isolated particles (pg per particle ($\pm$ s.d.)) was 0.008 ± 0.002 ; 0.031 ± 0.007 ; and 0.041 ± 0.007 respectively. The data represent average values of nine duplicate determinations of seven preparations.

When penicillin, tetracycline or chloramphenicol were added to the culture medium at the time of inoculation, the particles within the cells were destroyed and lost irreversibly from the cytoplasm. (Optimum concentrations that eliminated particles from the cytoplasm but did not significantly affect growth or division were 50 U ml^{-1} for penicillin, 0.5 $\mu\text{g ml}^{-1}$ for tetracycline and 0.25 $\mu\text{g ml}^{-1}$ for chloramphenicol.) Cycloheximide, neomycin sulphate and streptomycin had no such selective effect. Xenosomes *in situ* were also destroyed by irradiation with an electron beam (Fig. 2a): the effective dose (ED_{50}) was about 30,000 rad, comparable with values reported for X-irradiation of certain bacteria and members of the *Mycoplasma*¹¹. The ED_{50} for the protozoan itself was approximately 260,000 rad. Thus, xenosomes could be removed from the cytoplasm of the ciliates by treatment with the electron beam using doses of about 200,000 rad (see graph in Fig. 2a).

The infectious nature of the particles was demonstrated by adding aliquots of cell-free blendates or filtrates of xenosome-bearing cultures to particle-free animals and observing the uptake of the particles into the cytoplasm. The infective agent readily passed through Millipore filters with pores 8 μm

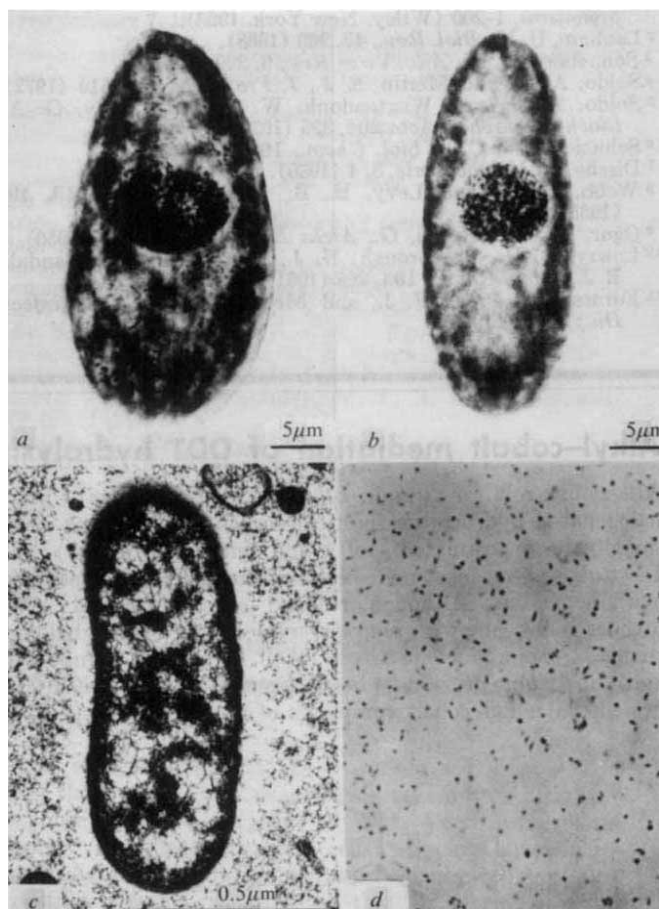


FIG. 1 Phase micrographs of xenosome-bearing (a) and xenosome-free (b) *Parauronema acutum* stained with aceto-orcein dye. (c), Electron micrograph of a xenosome *in situ*. Specimens were fixed in 1.33% OsO_4 buffered with 0.2 M γ -collidine, pH 7.4 prepared in seawater of density = 1.015 g cm^{-3} for 30 min at 0°C. Ciliates were left in buffer overnight at 0°C, dehydrated through an alcohol series and embedded in Araldite. Ultrathin sections were cut with a glass knife, doubly stained with alcohol-uranyl acetate and lead acetate and examined in a Phillips EM 300 electron microscope. d, Phase micrograph of isolated and purified xenosomes, unstained ($\times 570$).

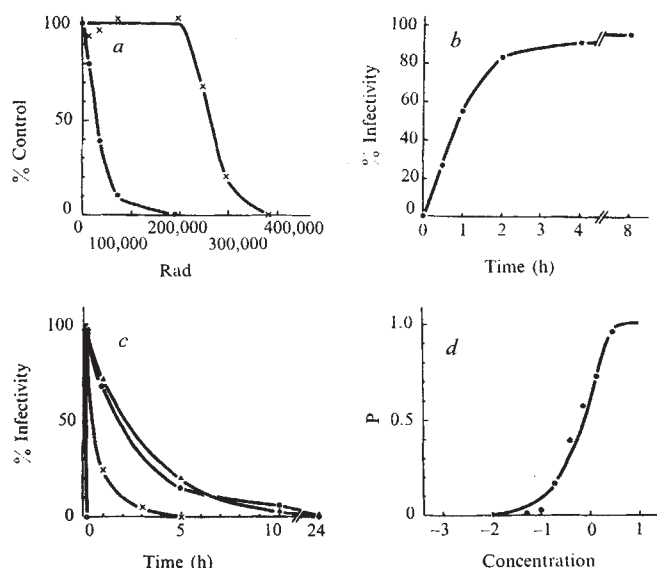


FIG. 2 *a*, Selective effect of electron beam irradiation on xenosomes *in situ*. *Xenosoma*-bearing *P. acutum* 110-3 was inoculated into 5 ml of fresh medium in sterile 30-ml tissue culture flasks (No. 3012, Falcon) at a cell density of $10,000 \text{ ml}^{-1}$. The cell suspension was 2 mm deep. Flasks were irradiated in a CLINAC 6 MeV linear accelerator (Varian Associates) with an output of 3.5 MeV at $10,000 \text{ rad min}^{-1}$ for doses up to 100,000 rad and $100,000 \text{ rad min}^{-1}$ for larger doses. Flasks were then incubated for 7 d at 22° as in the dark. Both protozoan and particle populations were estimated as described previously⁵. Population densities of non-irradiated protozoons averaged $2.3 \times 10^6 \text{ ml}^{-1}$; the number of xenosomes per protozoan was estimated at 204 ± 25 (s.d.). Thus, the 'population' of xenosomes calculated per ml of culture was 4.7×10^8 . x, Protozoan; ●, xenosomes. *b*, Release of infectious agent into ambient culture fluid. Exponential phase xenosome-bearing animals cultured in M medium were washed free of exogenous particles by centrifugation three times at $600 g$ for 1 min each, suspended in M medium at a cell density of $500,000 \text{ ml}^{-1}$ and left at 22°C in the dark. Samples were removed at intervals, filtered (Millipore, $8 \mu\text{m}$ pores) to remove the cells, and filtrates were assayed for infectivity as described in Table 1. *c*, Stability of infectious agent to temperature. Filtrates of exponential phase xenosome-bearing animals cultured in M medium were left at various temperatures. Samples were removed at intervals, brought rapidly to 22°C and assayed for infectivity (see Table 1) ○, 65°C ; ×, 37°C ; ●, 22°C ; ▲, 4°C . *d*, Experimental frequency of positive response (infectivity) to various concentrations of xenosomes in filtrates of xenosome-bearing *P. acutum*. Abscissa: logarithm of concentration (empirical scale). Ordinate: P , frequency of infection. Each point represents the average value of four experiments. The solid line was the curve derived from the expression $P_{\infty} = 1 - e^{-s}$ where P_{∞} is the probability of having at least one infective particle per protozoan and s is the average number of particles per sample. Infectivity was determined as described in Table 1.

Statistical analysis of the mode of action of the infectious agent present in culture filtrates suggests that a single particle can infect a susceptible particle-free animal (Fig. 2*d*).

Although host range studies were limited by the lack of well-characterised strains of marine ciliates, we have found that the xenosomes of this strain can infect three other particle-free stocks of *Parauronema acutum*, but not *Uronema nigricans* or the related *Parauronema virginianum*.

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Alkyl-cobalt mediation of DDT hydrolysis

A POSSIBLE role for vitamin B₁₂ in the detoxification of polyhalogenated hydrocarbon pesticides such as DDT has occasionally been postulated (ref 1 and references therein), and DDT has been shown to inhibit bacterial B₁₂-dependent methanogenesis². Although reduced cobalamins and cobaloximes will react with simpler species such as polyhalomethanes^{3,4}, no adduct between DDT or related compounds and cobalt has previously been observed². It has been suggested that alkyl-cobalt complexes of the ligand CR (Fig. 1)

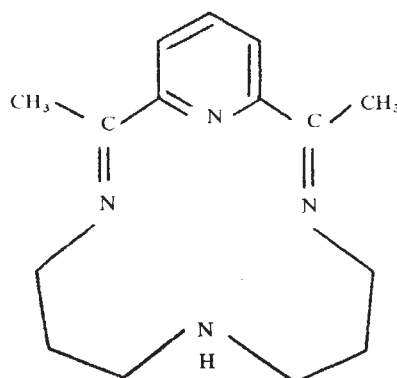


FIG. 1 The macrocyclic ligand CR.

in diameter, but not when the pores were $0.45 \mu\text{m}$ or less in diameter. We found no significant quantitative difference when whole filtered blendates were compared with cell-free culture filtrates for infectious activity (Table 1). This suggests that infectivity is associated with a particulate agent released from the protozoan into the culture medium. Figure 2*b* shows the release of the infective agent into the ambient culture fluid from particle-bearers washed free of exogenous particles. The infectious agent was relatively unstable to temperature (Fig. 2*c*). Infectious activity was lost almost immediately at 65°C ; about half the infectious activity remained after 1 h at 37°C and after 2 h at 22°C or 4°C . Sonication for periods as brief as 20 s completely inactivated particles present in cell-free culture filtrates. Attempts to stabilise the infectious agent by varying conditions of ionic strength, osmotic pressure and pH have been unsuccessful.

have some chemical similarities to B_{12} (ref. 5) and zinc complexes of CR have yielded results relevant to carbonic anhydrase⁶. We report the use of cobalt-CR to mediate the hydrolysis of DDT, the isolation of an alkyl-cobalt derivative of a hydrolysis product of DDT, and the formation of di(*p*-chlorophenyl)ketone from this compound.

The Co(II) complex of CR was prepared in aqueous ethanol by the standard template method⁷, and reduced *in situ* to the nucleophilic Co(I) species using borohydride at pH 9. This was allowed to react under nitrogen with pure *p*, *p'*-DDT, and the solution warmed to 40° C and stirred for 12 h in the dark. A deep red, light-sensitive solution resulted and addition of PF_6^- or ClO_4^- produced a red, tarry product. This was very difficult to purify but a small quantity of the ClO_4^- salt was isolated as a clean, maroon powder by repeated reprecipitation from ethanol/ether.

The infrared spectrum of this compound has an intense band around 1,100 cm^{-1} , confirming the presence of ClO_4^- . A broad area above 3,200 cm^{-1} and around the Nujol absorption at 2,900 cm^{-1} is attributable to a carboxylic acid group hydrogen bonding with water. The region 1,550–1,675 cm^{-1} is dominated by the C=C and C=N vibrations of the aromatic rings and these could well mask the C=O stretching vibration if this group displays some resonant single-bond character. The presence of ionic chloride in the compound was demonstrated by silver nitrate titration and the resulting solution was left in sunlight for a few days. Extraction of the solution with toluene yielded a white powder—shown to be pure by thin-layer chromatography—with a mass spectrum identical to that of an authentic sample of di(*p*-chlorophenyl)ketone. These results lead us to suggest that the complex is as shown in Fig. 2; this is supported by microanalysis (required percentages for $CoC_{20}H_{16}N_4O_4Cl_4$: C, 45.3; H, 4.6; N, 7.3; total Cl, 18.5; percentages found: C, 44.8; H, 4.5; N, 7.7; total Cl 18.1) and by mass spectrometry. Thermolysis of the compound on the mass spectrometer probe at 200° C produced many fragments but, considering only those displaying peaks at the highest values of m/e , groups are observed at $m/e = 248, 250, 252$ (intensities 9:6:1) and 235, 237, 239 (9:6:1). The isotopic distribution of ^{35}Cl and ^{37}Cl implies that these peaks are due to species each containing two chlorine atoms. Since the aromatic chlorines will be stable under the reaction conditions used, in contrast to the aliphatic chlorines, we assign these peaks to $(p-ClC_6H_4)_2C_2H_3^+$ and $(p-ClC_6H_4)_2CH^+$. The latter is expected from cobalt-carbon bond cleavage and loss of CO_2 , and peaks attributable to $(p-ClC_6H_4)_2CH.C(OH)_2^+$ are present in the region m/e 281–285 but the relative intensities are obscured by the presence of other fragments. It has been pointed out to us that the former set of peaks may be due to hydrogen transfer from the CR ligand during thermolysis.

The formation of the isolated compound from Co(I)CR and DDT may be readily rationalised. Nucleophilic attack by Co(I) will displace Cl^- and the resulting complex can then eliminate H^+ and Co(I) to form 1,1-di(*p*- ClC_6H_4)-2,2-

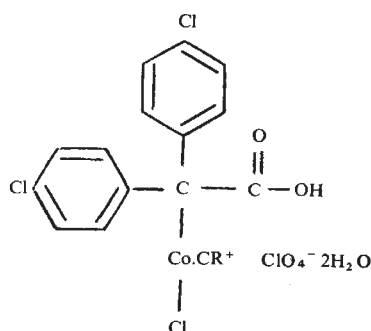


Fig. 2 Suggested structure of the isolated alkyl-cobalt intermediate.

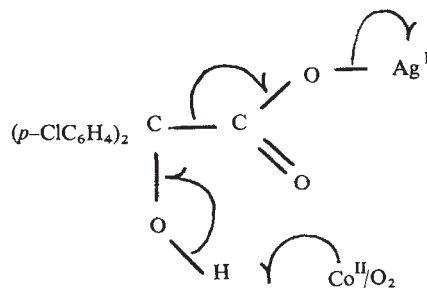


Fig. 3 Suggested decarboxylation mechanism.

dichloroethylene; further attack on C1 by Co(I) and hydrolysis of the chlorines on C2 gives the aldehyde hydrate which is easily oxidised to the acid during workup. The type of reactions involved here have been fully documented by Schrauzer^{4,8}. The production of di(*p*-chlorophenyl)ketone is explained by photolysis of the cobalt-carbon bond to form a radical, which in aqueous solution gives rise to the α -hydroxy-carboxylic acid. In the presence of Ag(I) and a radical species such as Co(II) or O_2 this will readily decarboxylate to the ketone (Fig. 3) (J. Staunton, personal communication).

These results indicate that the involvement of alkyl-cobalt species in the hydrolytic detoxification of DDT is chemically feasible. They also show that hydrolysis and partial degradation of DDT by this route requires far less vigorous conditions than the strong base and oxidising agents usually used to bring about these reactions. There are evidently many possible reaction pathways for the dechlorination of DDT in complex biological systems such as anaerobic sewage sludge⁹. The presence of microorganisms in these wastes means that B_{12} -mediated routes are available—several such routes have also been implicated in the biological fixation of mercury^{10,11}—and it is thus of great interest to note that di(*p*-chlorophenyl)ketone is formed among other products from DDT in sewage sludge¹². The possible involvement of B_{12} in the formation of this compound, rather than its being a degradation product of di(*p*-chlorophenyl)acetonitrile¹², is open to consideration.

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GENERAL

Genetics and the development of social attitudes

WHATEVER the current norms might be with regard to public opinion about various issues, and whatever factors contribute to their change with time, attitudes are far from uniform. Eysenck¹ has shown how responses to a public opinion questionnaire could be resolved into two main factors: 'radicalism' (*R*) against 'conservatism' and 'toughmindedness' (*T*) against 'tendermindedness'. Individual differences in opinion are, to some degree, the social manifestation of individual differences in personality. Significant covariation has been detected between various measures of extraversion (*E*) and *T*. Wilson² has suggested personality correlates of *R*. Such relationships, together with available evidence for the genetic determination of personality differences (see, for example, ref. 3), justify a more penetrating analysis of the extent to which variation in opinion and personality share a common genetic basis.

We consider the causes of trait covariation within and between pairs of monozygotic (MZ) and dizygotic (DZ) twins. From the responses of 708 volunteer pairs of like-sexed twins to an 80-item Personality Inventory and to a 60-item Public Opinion Inventory, scores were derived on scales of psychoticism (*P*), extraversion (*E*), neuroticism (*N*), *R* and *T*, together with an emphasis (*Em*) score which indicated any tendency of subjects to adopt more extreme opinions. The questionnaires and the scoring keys used have been published elsewhere^{1,4}.

On the basis of replies to questions concerning childhood similarity, the twins were diagnosed as MZ or DZ (ref. 5). The pairs differed in their ages and in the time for which they had lived apart. Our analysis is based on the raw scores and the absence of an age correction will not severely affect the conclusions from a study which can only detect gross effects.

The MZ intrapair differences in personality were not related to the duration of separation, so we have pooled separated and unseparated twins. The composition of the sample by zygosity and sex is shown in Table 1, and it can be seen that it is not representative of zygosity or sex, although the twins are representative of the population with respect to variation for the traits in question. The data were summarised by the mean squares and mean products within and between pairs for every trait and group of twins.

Twin data alone are inadequate for a detailed genetic analysis, particularly when the twins were not properly separated⁶, so the structure of our data limited us to tests of comparatively simple hypotheses. We had to accept estimates of components which may be biased and tests which are not very powerful.

We wished to test, and hoped to choose between, two hypotheses of equal rank about the causes of variation and covariation for our six scales of personality and attitude. The hypotheses are: (1) that all the observed trait variation and covariation is attributable either to differences between the environmental influences shared by members of the same pair (E_2), or to differences in the environmental influences specific to individuals within the family (E_1); and (2) that all the variation and covariation is attributable either to the cumulative and additive effects of many loci for which the population is polymorphic (D_R), or to specific (E_1) environmental influences. We refer to the first hypothesis as the 'simple environmental hypothesis' and the second as the 'simple genetic hypothesis'.

For a particular pair of traits, *i* and *j*, we can write expectations for the mean products, in terms of the genetic and environmental covariance components for that pair of traits.

TABLE 1 Composition of the sample of twins (number of pairs)

Zygosity	Sex		Total
	Male	Female	
MZ	120	331	451
DZ	59	198	257
Total	179	529	708

These components are D_{Rij} , E_{1ij} and E_{2ij} , and they have been defined in detail and the restrictions on their interpretation discussed elsewhere¹. The expectations derived for both simple hypotheses are given in Table 2.

The coefficients of D_R are applicable to randomly mating populations. The slight assortative mating observed for personality variables is insufficient to cause a serious departure from these expectations, since the heritability of the traits is quite low. Insel⁸ reported correlations between spouses for the Wilson Conservatism Scale, finding values between 0.59 and 0.66. If this correlation had a large genetic component, its effect in our case, would be inseparable from E_2 and would contribute to failure of our simple genetic model. It is possible to obtain weighted least squares estimates of the parameters of either model⁷. A detailed analysis suggested that the genetic and environmental parameters might be inconsistent over sexes. At the risk of introducing some redundancy into our model, therefore, we allowed different values for our estimates in the two sexes and subsequently compared them. There are 168 raw statistics altogether, and we fit 84 parameters, leaving 84 d.f. for testing either model.

When the simple environmental hypothesis was tested we found that the residual $\chi^2_{84} = 167.31$ ($P < 10^{-6}$) so we concluded that a model invoking only environmental factors fails to provide an adequate description of the variation and covariation of the six traits. For the simple genetic hypothesis, we found that the residual chi square is smaller ($\chi^2_{84} = 111.60$, $P \approx 0.025$) and just significant. A model in which D_R , E_1 and E_2 were fitted jointly gave a very satisfactory fit ($\chi^2_{84} = 47.98$, $P \approx 0.24$), suggesting that some E_2 parameters might be added to our simple genetic model. By this stage, however, there were many redundant parameters in the model and in the absence of any theory about which parameters could be deleted from the more complex model, we present the results of the simple genetic model, bearing in mind that some modification might still be appropriate (Table 3). The results were obtained by pooling estimates over sexes.

The estimates for the components of variance can be used to calculate heritabilities for the six traits from

$$h^2 = \frac{1}{2} \hat{D}_R / (\frac{1}{2} \hat{D}_R + \hat{E}_1)$$

and provided we are justified in accepting our model, these estimates refer to the proportion of the population variance which is genetic in origin (Table 4).

The simple environmental model is insufficient to explain the observed variation and covariation of the traits in this study. We are, however, restricted by the structure of the data available. A larger or better designed study might identify common environmental influences in the departures from expectation which, in our study, appear as error. We

TABLE 2 Expectations of mean squares (mean products) on two simple models

Model Parameter	Expectation		Genetic	
	Environmental E_{1ij}	E_{2ij}	D_{Rij}	E_{1ij}
Mean square/product				
Between MZ pairs	1	2	1	1
Within MZ pairs	1			1
Between DZ pairs	1	2	$\frac{3}{4}$	1
Within DZ pairs	1		$\frac{1}{4}$	1

TABLE 3 Estimates of parameters of a simple genetic model*

Scale	<i>R</i>	<i>T</i>	<i>D_R</i> (genetic)		<i>E</i>	<i>N</i>	<i>R</i>	<i>T</i>	<i>E₁</i> (environmental)		<i>E</i>	<i>N</i>
			<i>Em</i>	<i>P</i>					<i>Em</i>	<i>P</i>		
<i>R</i>	12.39¶	-1.43	4.38§	0.81	-0.29	2.08	3.37¶	-1.19¶	1.37‡	0.02	0.03	0.19
<i>T</i>		‡21.53¶	-4.19	2.11§	6.62¶	2.61		‡9.18¶	-2.67§	0.71§	-0.32	0.06
<i>Em</i>			40.67¶	2.47‡	-0.82	2.85			34.76¶	0.44	1.65	1.73
<i>P</i>				3.19¶	0.48	1.80‡				2.92¶	0.67‡	0.05
<i>E</i>					17.61¶	-1.21					9.41¶	-0.13
<i>N</i>						22.04¶						11.25¶

* Values given after pooling over sexes. Where the estimates were heterogeneous over sexes they are marked †.

‡ Significant at the 0.05 level.

§ Significant at the 0.01 level.

¶ Significant at the 0.001 level.

can, however, be reasonably confident that some kind of genetic mechanism has to be invoked to account for the observations so far. That such a mechanism might involve the correlation of developmentally significant environmental factors with genetic variation should not be discounted. About half the variation between individuals for the six traits has a genetic basis (Table 4).

There are also some predictable significant covariance terms. Our study confirms the correlation between *E* and *T*, showing that it has a genetic, rather than an environmental basis, since only the *D_R* component is significant. There is also a significant genetic correlation between *P* and *Em*. This suggests that individuals who achieve high *P* scores share the strong conviction of being right, which is characteristic of some psychotic behaviour. The more significant tendency, for high *P* scores to be associated with 'toughmindedness' scores, is perhaps indicative of psychopathic tendencies which are known to be related genetically to psychosis and hence to *P* (ref. 9).

Although we claim to show some common basis for differences in personality and attitude, we stress that, for the scales in question, the greater part of the genetic and environmental variation is specific to particular traits. 'Radicalism' scores, for example, are totally independent of the measured personality variables, although they have significant associations with the other attitude measures.

Genotype-environmental interaction could inflate our estimates of both *D_R* and *E₁*. Some kinds of correlation between *G* and *E* may be discounted because the fit of the *D_R*, *E₁*, *E₂* model implies that the total dispersions are homogeneous for the different kinds of twins. In many cases, however, genotype-environment correlation, if present, remains as a source of bias in our estimated genetic components.

Our model assumed that gene action is purely additive. Even without the complication of *E₂* it would have been virtually impossible to identify any non-additive variation in our study. The bias in parameters estimated from twin studies in the presence of dominance is discussed elsewhere⁷.

Extrapolation from these results to other populations and cultures is likely to be misleading because they are almost certain to differ with respect to the relative importance of different determinants of variation.

If we restrict ourselves to the British population and contemporary culture, we expect, on the basis of our simple

genetic model, that attitudes in parents would be relatively poor predictors of attitudes in offspring. Parent-offspring correlations would be about $\frac{1}{2}ht$, given random mating, and thus to be roughly between 0.2 and 0.3. Many of the available familial correlations for attitudes (see for example, ref. 10) come from pre-war studies and show parent-child and sibling correlations which are much higher. The difference, if real, could reflect the consequences of cultural change during the last 30-40 yr. If individuality and mobility were not encouraged, common environmental (*E₂*) influences might play a significant part in maintaining the pattern of variation in attitude. In such a situation, parent-offspring correlations would resemble those for siblings and MZ twins. A culture which promotes individuality and mobility, however, may reveal a pattern of similarity between relatives which depends more obviously on genetic components and give greater significance to the environmental experiences which are unique to the individual, irrespective of his family environment. More recently, Insel⁸ has obtained correlations between parents and offspring for the Wilson Conservatism Scale which are still too large to be consistent with a simple genetic model, so we cannot regard the issue as settled. The sensitivity of attitudes to cultural change may, however, make them a useful indication of the interdependence of gene expression and culture and encourage further research in this subject.

The computations were conducted on the University of Birmingham KDF-9 computer. We thank the Social Science Research Council and the Medical Research Council for grants, Mrs J. Kasriel for help in data collection, and Professor J. L. Jinks for helpful comments on the manuscript.

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TABLE 4 Heritability estimates for personality and social attitude scales

Scale	<i>h²</i>
<i>R</i>	0.65
<i>T</i>	0.54*
<i>Em</i>	0.37
<i>P</i>	0.35
<i>E</i>	0.48
<i>N</i>	0.49

* Based on pooling heterogeneous estimates for two sexes.

Persistence of traditional beliefs among undergraduates

JAHODA¹, Pasachoff *et al.*², and Salter and Routledge³ have investigated the hypothesis that university education leads to the decline of supernatural beliefs, arriving at strikingly similar results that contradict the hypothesis. We carried out an extension of these studies at our institution. Our investigations were aimed at an evaluation of the persistence of traditional, Eastern beliefs among undergraduates.

One hundred and forty science undergraduates (100 male and 40 female), chosen at random, answered 14 questions on astronomy, astrology, superstitions, evil spirits, auspicious time, pilgrimage and sacred animals (Table 1).

The returned questionnaires were first processed by the year of study and then by sex. The options to each question—certainly believe, sometimes yes, sometimes no and certainly do not—were allotted scores of 20, 11, 9 and 0 respectively (as in ref. 1 where 0 denoted 'total unqualified disbelief' and 20, 'total unqualified belief'). For each question these scores were summed and then the sum was divided by the number of respondents, giving a 'mean belief score'. There are two questions for each topic so by averaging the

TABLE 1 The questionnaire

This study seeks to evaluate the degree of traditional beliefs held by the students of our college. The answers shall be treated as confidential and it does not seek to evaluate individual students. Each of the questions below are provided with four options. Please indicate clearly by an 'X' the option most relevant to you.

Personal data:

- (i) Year of graduate study: 1 yr, 2 yr or 3 yr.
(ii) Sex: male or female.

The respondents were then asked to answer certainly believe, sometimes yes, sometimes no or certainly do not to the following questions.

Astronomy:

1. Do you believe that the stars and planets have any influence over your daily routine?
2. Do you believe that the appearance of a comet or an eclipse brings bad luck?

Astrology:

3. Do you believe in consulting your partner's horoscope before settling for marriage?
4. Do you believe in palmistry and numerology?

Superstitions:

5. Do you believe that Friday, the 13th, is an unlucky day?
6. Do you believe that treading on one's nails would bring misfortune?

Evil spirits:

7. Do you believe that evil spirits dwell in the grave yard?
8. Do you believe in the power of witchcraft and charms?

Auspicious time:

9. Do you believe in an "auspicious time" before setting off on important ventures?
10. Do you believe that a child must be born at an "auspicious time" to be successful in life?

Pilgrimage:

11. Do you believe that the River Ganges has the ability to wash away your sins?
12. Do you believe that shaving your head fulfills your vow to God?

Sacred animals:

13. Do you believe that the peacock (bird) is a vehicle of God?
14. Do you hold snakes and cows to be sacred animals?

TABLE 2 Average belief scores by the year of graduate study

Question	First year	Second year	Third year	All years
Astrology	7.67	7.43	7.37	7.49
Astronomy	6.05	5.96	5.85	5.96
Superstitions	8.48	8.16	8.11	8.25
Evil spirits	7.77	7.75	7.73	7.75
Auspicious time	9.99	9.72	9.42	9.71
Pilgrimage	7.01	6.95	6.83	6.93
Sacred animals	7.07	6.95	6.62	6.88
Mean	7.72	7.56	7.42	7.567
No. of subjects	42	41	57	140

two mean scores an 'average belief score' for that topic is obtained.

A score of 5 or above was taken to indicate a persistence of beliefs. For example, with the students overall, we found an average belief score of 5.96 for the traditions in astronomy and 9.71 for auspicious time (Table 2), indicating a greater persistence of the belief in auspicious time over the astronomical traditions.

The mean score for all students and for all the questions is 7.567, indicating the prevalence of traditional, Eastern beliefs to a marked degree among science undergraduates (Table 2). But, unlike the study at the University of Pennsylvania³, there seems to be a difference in attitude between the sexes (Table 3). This may be attributable to both the environment and sociology of Indian society. Table 2 also shows a very gradual decline in beliefs with increasing time spent at the university.

TABLE 3 Average belief scores by sex

Question	Male	Female	Total
Astrology	6.77	8.21	7.46
Astronomy	5.81	6.11	5.96
Superstitions	8.16	8.34	8.25
Evil spirits	7.21	8.29	7.75
Auspicious time	9.36	10.06	9.71
Pilgrimage	6.84	7.02	6.93
Sacred animals	5.23	8.53	6.88
Mean	7.054	8.08	7.567
No. of subjects	100	40	140

High scores of 9.71 and 8.25 for auspicious time and superstitions, respectively, may reflect the deep-rooted nature of these beliefs. It was surprising that a mean score of 5.96 was recorded for astronomy, despite it being a finite science. The vast difference in scores for sacred animals between the sexes seems to be an example of the sex-differentiated attitude towards these beliefs.

In conclusion, although our study was limited to science undergraduates, the results are similar to earlier studies¹⁻³. If traditional beliefs hinder scientific training, a way must be sought for their eradication to improve our activities in science.

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book reviews

Indian view of research

Economics of Research and Development. By P. M. Chowdhury. Pp. xii+163. (People's Publishing House: New Delhi, (1973.) Rs18.

At a time when national expenditure on research in the United Kingdom has declined from the vigorous growth rates of the past two decades to the current provision which barely maintains the present level of activities there is a great deal of thought about the "economics of R and D". Mr Chowdhury's subject is therefore timely.

The publication, in one volume, of eleven essays chosen by the author from a decade of his writing "about the science of science" does not have the cohesion and logical development of thought which this difficult subject undoubtedly needs. Readers will wish that the author had found the time to condense some of the more generalised essays and to link to them more coherently. He does not communicate ideas very easily and has a rather irritating habit of generalising in the conditional tense, often using technical slang unnecessarily, but the persevering reader is rewarded with nuggets of down-to-earth commonsense and useful ideas.

Those who seek methods for the evaluation of any wide-ranging national programme of research will readily agree that there are "woeful gaps in the data system" and will confirm the difficulties of quantifying research output in terms of the production of scientific papers or of the registration of patents. Few of Mr Chowdhury's scientific readers are likely to share his confident assertion that the economist is nonetheless able to select research priorities and to assess research programmes.

In the early essays it becomes clear that the author is concerned mainly with industrial research, that is with product and process studies, which are more amenable to short-range planning and assessment than are the endless surprises of the biological world in agricultural research. The part which the author considers chance to play in research is revealing in this context, and most experienced research workers will be startled to read that "it is difficult to obviate the chance factor in research" and that "it has been assumed that the impact of the chance factor in R and D sector is more or less on par with that originating in the

production process in other sectors". Emphasising the greater role of state involvement in R and D in developing, as compared with developed countries, the author concentrates heavily on the industrial sector and pays too little attention to the predominantly agricultural occupations of his fellow-countrymen. In both the United States and the United Kingdom the state involvement in agricultural R and D outweighs that of the private sector.

Mr Chowdhury takes a somewhat unflattering view of scientific research workers. The image of a research manager as one who "assumes the role of an erudite scientist, an efficient organiser or a smart salesman" would startle many senior scientific administrators, but it is uncomfortably near to the truth.

These essays show that India has encountered very much the same underlying problems in the organisation of science as have the more intensively developed countries, for example that "public sector research is structurally separated from industry". Indeed the major part of the reorganisation of British public research on the basis of Lord Rothschild's advice, in three major aspects of biology, those of medicine, agriculture and the natural environment, was to overcome this separation in a highly developed economy. Mr Chowdhury's solutions are similar to those of other countries: of technically efficient scientific advisory committees, with a large measure of independence and a long and stable lease of life. The British Industrial Research Associations would be in full agreement with the author's conclusion that "sponsorship of projects by industries may make them more conscious about programming aspect of the project".

The author concludes that in many developing countries research projects of national importance have failed to receive sufficient momentum for want of really capable personnel for managing them, which summarises effectively some two decades of premature campaigns for "localisation".

The eleven essays offer comments on planning, investment and programming in R and D, balance of payments and conservation of foreign exchange, employment of scientific and technical personnel and the perspectives of development.

In a brief but illuminating foreword by Dr B. D. Nag Chaudhuri, Director

General of R and D in the Ministry of Defence, there is set out very clearly the dilemma of Indian scientists, cut off by their higher standard of living from the largely agricultural problems of the population, but uneasy in their uncertain relationships with the all-powerful administration.

These essays will interest all those concerned with developing countries and should be particularly valuable for those who strive to understand the vast complexities of the modern Indian scene.

H. C. PEREIRA

The poetical chemist

The Wild Uncharted Country. By Deric Bolton. Pp. 48. (Outposts: Walton-on-Thames, Surrey, 1973.) 75p.

SCIENTIFIC autobiography in blank verse is a literary genre not often attempted, but this little book shows that it can be done successfully. Although the author's note tells us that the events described are imaginary, the links with real life are close. The protagonist of the poem (he is too unheroic to be called the hero) struggles to qualify at night school; then he has a spell of chemical research at South Kensington; enters industry in the 1930s; engages in "vital war work"; returns to the chemical industry after the war; retires in the 60s; and is now concerned with environmental and ecological issues, after a lifetime as

An ignorant chemist whose ways of life had run

Within deep narrow channels.

His life has flowed like a river, carrying him he knows not where; his career proceeded without self-guidance, seemingly by blind chance. After he retired, the stream broadened out into a flat expanse, letting him see at last the lie of the land around. The poem gains strength from our familiarity with this Neoplatonic imagery of the individual being borne as in a boat down the turbulent stream of life, used directly in Shelley's *Alastor* and obliquely in Coleridge's *Ancient Mariner*.

A poem on so mundane a topic depends crucially on the quality of the wording, a challenge which the author meets with a fair degree of success. The writing is careful and, although there are some dull patches, the narrative flows easily and has enough imaginative moments to hold the attention. The chief flaw is that the chemist himself is too much of a puppet, with

little will of his own and no private life (or none that is mentioned). But his 'discovery' of ecology at the end brings a touch of strong, almost Wordsworthian feeling:

So, finally I chiselled out this goal:
To learn to live with nature,
not oppose it,
Exploit, burn up and devastate:
To change from miners into
farmers . . .
. . . crashing down those barriers
which divide
Science from man and man from
natural
Things and man from man and all
that is
Of simple panoramic love and
permanence.

D. G. KING-HELE

Dangerous insects

Insects and Other Arthropods of Medical Importance. Edited by Kenneth G. V. Smith. Pp. xiv+561. +12 plates. (British Museum (Natural History): London, 1973.) £6.50.

THIS is a revised and expanded edition of an earlier book by Dr J. Smart, published in 1944, which dealt mainly with the fauna of the Old World and included little biological information. The present work is much larger, covers the whole world and is by 16 authors. The primary purpose of the book is still to enable non-specialists to identify to generic or subgeneric level arthropods, especially insects, of medical importance. Many species which are essentially parasites of animals are also included because many of them also attack man, if only on rare occasions. It will probably come as a surprise to the non-specialist to learn that several moths feed on eye liquids of vertebrates and that one is even capable of piercing the skin and sucking blood.

Three hundred of the 523 pages are devoted to the order Diptera whose importance has been greatly increased by the recognition of many new viruses. Of the 553 different combinations of vector and disease listed in their tables summarising the records, 10 involve bacteria, 47 nematodes, 90 protozoa, 11 rickettsias, 9 spirochaetes and 386 viruses (mostly carried by mosquitoes and recognised in the past 30 years). Many of these viruses are, of course, very localised and often quite unimportant and the long known diseases such as malaria, plague and typhus are much more serious. Nevertheless, the only arthropod groups of much importance in disease transmission besides the Diptera are the Siphonaptera (fleas)

and the Acari (ticks and mites). Though disease transmission is the chief way in which insects are medically important, they may also be troublesome by biting or stinging, or by fragmenting into particles which are irritating or to which man may become allergic. Even scorpions which are relatively rare may kill 1,000 people, mostly young children, each year in Mexico.

The illustrated keys for the identification of all the more important groups are excellent and will be very useful to anyone with some knowledge of entomology. In most groups, there are also keys for the identification of the early stages (larvae and pupae). The information about the biology of the various groups and of the kind of trouble they cause is concise but sufficient, together with the long lists of references, to give a useful introduction to the subject.

Readers should note that a short mimeographed list of errata has been inserted in each copy. It is chiefly concerned with some errors in the names of groups of Acari in the table of disease vectors.

O. W. RICHARDS

Halides galore

The Chemistry of the Carbon-Halogen Bond. Edited by Saul Patai. Part 1: Pp. xiii+1-608; part 2: Pp. xiii+609-1215. (Wiley-Interscience: London and New York, December 1973.) £22 (two volumes).

Fluorine in Organic Chemistry. By R. D. Chambers. Pp. xv+391. (Interscience Monographs on Organic Chemistry.) (Wiley-Interscience: New York and London, January 1974.) £10.75.

THE first monograph deals with the thirteenth functional group to appear in Professor Saul Patai's monumental series, now within sight of completion. As with previous volumes, editorial policy has been to go to press without promised chapters that had not been received by a deadline. This time there are five casualties: formation of C-X bond; modern synthetic uses of organic halides; synthesis and uses of isotopically labelled halides; fluorocarbons (see below); and, finally, optical rotatory dispersion and circular dichroism of organic halides. Of these the first two are losses indeed.

There are, however, plenty of interesting and valuable articles in what remains. Among them are: heterolytic mechanisms of substitution involving carbon-halogen bonds (P. B. D. de la Mare and B. E. Swedlund, 141 pages); directing, activating and deactivating effects (G. Modena and G. Scorrano, 106 pages); elimination reactions in solution (R. A. More O'Ferrall, 68

pages) three excellent reviews on major aspects of the subject that are of general interest. There is also an interesting chapter on homolytic mechanisms of substitution (E. S. Huyser, 60 pages), but as this deals entirely with C-H→C-X, it seems to belong more appropriately under alkanes. Then there is a series of chapters dealing with more specialist aspects of the C-X linkage: mass spectrometry (A. G. Loudon, 42 pages), pyrolysis (K. W. Egger and A. T. Cocks, 70 pages), photochemistry (P. G. Sammes, 48 pages), radiation chemistry (R. E. Bühler, 70 pages), biochemistry (S. Doonan, 52 pages), electrochemistry (J. Casanova and L. Ebersson, 70 pages) and thermochemistry (R. Shaw, 23 pages).

There is a very long chapter on analysis of organic halogen compounds (J. Zabicky and S. Ehrlich-Rogozinski, 160 pages) which, though interesting, seems to be overweight compared with space devoted to other topics. There are the usual introductory chapters on general and theoretical aspects of the carbon-halogen bond (G. H. Wagnière, 48 pages) and structural chemistry of the C-X bond (J. Trotter, 14 pages); and also one on hydrogen bonding and complex-forming properties (J. W. Smith, 36 pages). Then there are the topics that don't quite fit in anywhere else; perchloro-, perbromo- and periodo-compounds (T. Chivers, 62 pages), and, last of all, rearrangements involving halides (C. Raape, 44 pages—an interesting chapter with a good account of Favorskii and Ramberg-Bäcklund rearrangements).

All in all a good volume, though as with previous volumes one is often puzzled at the way in which space is allocated between the various topics. The reproduction of structural formulae is in general fairly good, and the layout relatively clear. Literature coverage seems to be very thorough, but to extend only into 1971 (the end?), with some citations extending into 1972 in a few articles.

The non-delivery of the fluorocarbon chapter in Professor Patai's collection is more than compensated for by the appearance of Dr Chambers' book. This does not set out to provide synoptic coverage of the subject, but seeks to place selective aspects in a general mechanistic framework, for example, total, and selected, fluorination; replacement of OH by F; nucleophilic displacement of F; eliminations; polyfluoroaliphatics and aromatics; organometallic compounds. It is an interesting, useful and well documented survey, but the author is ill served by bad layout and by poor unattractive representation of reaction schemes. Literature coverage is essentially till the end of 1971.

PETER SYKES

Crystal defects

Computed Electron Micrographs and Defect Identification. By A. K. Head, P. Humble, L. M. Clarebrough, A. J. Morton, and C. T. Forwood. Pp. x+400. Defects in Crystalline Solids, vol. 7. (North-Holland: Amsterdam and London; American Elsevier, New York, 1973.) Dfl 100; \$35.10.

THE interpretation of electron micrographs of thin foils of crystalline material involves two radically different aspects: first, the morphology and orientation relationships of features such as precipitates, transition phases (for example, Guinier-Preston zones) and twins; second, the geometrical characteristics of crystal defects such as dislocations, stacking faults and point-defect aggregates. The first task involves simple examination of micrographs and matching of the diffraction patterns of selected areas, and offers no particular challenges to the experienced microscopist; the second is far more difficult, since the image (for instance) of a dislocation is really the image of a local distortion in the crystal lattice, and to identify the geometrical characteristics of the defect (Burgers vector, stacking parameters, and so on) requires the investigator to deduce the details of the lattice distortion from the appearance of the image under different imaging conditions. In fact, several images of the same defect are needed to interpret it securely.

The established way of identifying the Burgers vector of a dislocation is to find an imaging mode which causes the dislocation to disappear, but this method has limitations, especially with elastically anisotropic materials. The other method involves generating a theoretical image, with the aid of a computer program and pictorial print-out, for a particular set of imaging conditions, and comparing the theoretical image critically with that photographed in the electron microscope.

This book is the first detailed presentation of this computer technique, by a team of Australian authors led by A. K. Head, who originated the technique seven years ago. The book includes an outline of the two-beam dynamical theory which is the theoretical basis of the method; a full account, with many examples, of the practical steps involved in orienting a crystal, identifying the diffraction vector, dislocation line direction, and so on; the principles and details of the several computer programs, for both cubic and non-cubic crystals; and the methods for matching experimental and theoretical images. Finally the uses and limitations of the method are outlined.

The book is very much a practical operator's handbook; while its main use of course is to show the reader how to compute images, it will also be useful for the

beginner who is learning how to orient foils and features in foils. Altogether, this is an impressive compilation by a group of pioneers who have stayed the course and finished the job.

R. W. CAHN

Quaternary fossil plants

Quaternary Plant Ecology. Edited by B. J. B. Birks and R. G. West. (The 14th Symposium of The British Ecological Society, University of Cambridge, 28-30 March, 1972.) Pp. ix+326. (Blackwell Scientific: Oxford and London, 1973.) £13.

THE relation between the ecology of plants, as seen today, and the study of fossil assemblages of plant remains from the Quaternary period has always been an exchange of information and ideas in both directions. It is generally realised that any attempt to interpret what an assemblage of fossil remains really represents, must depend on a knowledge and understanding of modern vegetation and the ecology of the constituent species. In return, the study of fossil assemblages leads to a much better appreciation of the extent to which the composition of present day vegetation depends on its history, both directly and indirectly through concurrent changes in the soil. The papers presented at this symposium are primarily concerned with the first relationship, that is, the ecology of the past but quite clearly a better knowledge of the past helps understanding of the present.

The first four sections, containing eleven papers, are directly concerned with methodology and the process of matching assemblages of pollen, or in one paper, other fossilised parts of plants, with modern vegetation. It is at once clear that a technological revolution has taken place as a consequence of the use of the proportions of carbon isotopes to give an independent estimate of the absolute age of a deposit. This allows the rate of deposition of a sediment to be calculated and thus pollen contents can be measured in absolute, rather than relative terms. This has stimulated a much more fundamental approach to the problem of relating the quantity of pollen deposited on unit area in unit time to the actual representation of species in the surrounding vegetation. Studies of the production of pollen, its dispersal in the air, its redistribution in water and its preservation are all included. These sections provide a fund of information and ideas which should be read by all palynologists.

The fifth section contains four papers concerned with the evidence from quaternary studies of the rates of change in vegetation over long periods, including both natural processes and changes for which man is largely responsible. The

sixth section is devoted to limnological studies which demonstrate the value of interdisciplinary studies using palynology and stratigraphic analysis of a variety of aquatic organisms.

Symposia tend to be rather uneven in their coverage and often include papers of relatively light weight. The very opposite seems to be true in this case. Those who planned, organised and reported the meeting are to be congratulated on bringing together so many stars each of such magnitude into a single constellation and on producing an outstanding contribution to the literature of palaeoecology.

C. D. PIGOTT

Eccentric extracts

Strange Phenomena: A Sourcebook of Unusual Natural Phenomena, Vol. G-1. Compiled by William R. Corliss. Pp. 277. (Corliss: Glen Arm, Md 21057, 1974.) \$6.95.

As the flow of letters we receive whenever *Nature* publishes anything about ball lightning testifies many of our readers are interested in bizarre phenomena at the fringe of scientific understanding. That is as it should be; only by such interest can any of these phenomena be brought within the fold of science proper, just as continental drift has gained respectability during this century. So the publication of this sourcebook is likely to meet with a warm response.

Corliss has collected what he calls "homeless facts" and given them a home, in the form of a collection of brief reports (some no more than 200 words) culled from the scientific, pseudo-scientific and unscientific literature. The task is far from complete, and we are promised further volumes as the work progresses; the present edition is in the form of a ring-binder, so that necessary additions or deletions can be made conveniently.

Some examples culled from a random search of the pages suffice to give an impression of the book's scope: ball lightning, flames from the sky, earthquakes and lightning, strange detonations (barisal guns and the like), sunspots and rainfall, and so on. The march of science is such that the last of these phenomena has already been received into the bosom of 'serious' science, and most of the oddities which are mentioned are far from the lunatic fringe of flying saucers and little green men.

The net cast by Corliss has gathered a harvest from many sources, and *Nature* is lavishly represented. But it is a curious feature of the extracts from this journal in particular that they are clustered distinctly in time about two peaks, the first about 100 years ago and

the second in the past few years. This may reveal something about the collection technique used by Corliss, or about this journal, or about the general awareness of such phenomena and 'things that go bump in the night'; I doubt if it reflects a genuine distribution of such oddities. But the discovery of such a pattern shows just one example of the way in which the book can provide hours of harmless fun. At the price, it is excellent value for entertainment alone; there is also the added bonus that such a collection must inevitably include something which will become 'reputable' in the near future. Owners of the volume will then be able to say "Of course, I knew all about that back in 1974".

JOHN GRIBBIN

Plan the countryside

Rural Resource Development. By M. C. Whitby, D. L. J. Robins, A. W. Tansey and K. G. Willis. Pp. 243 (Methuen: London, January 1974.) £2.50 cloth; £1.30 paper.

THE main concern of the authors is to present ways of analysis which will lead to more effective public decision making and lead to the better use of rural resources. At present many of the decisions affecting the countryside are reached in an illogical way; because recommendations at officer level concentrate on only a selected scheme, because of political considerations, because of the influence and pressure of organisations such as the Country Landowners' Association, the National Farmers' Union, and strong amenity bodies.

This book gives the elements of the economic theory of resource allocation to provide a framework for understanding the economic processes at work in the countryside; private rural economy is well documented whereas public issues have received less attention. The authors explore in some detail the factors which do, as well as those which should, lead to decisions. They contend that most public agencies seem to pursue inappropriate objectives under incorrectly specified constraints, and that suboptimal decisions are taken because cash revenue and expenditure are used instead of the assessment of social costs and benefits.

There is a chapter on recreation, conservation and amenity which explores the general question of allocating land to physically non-productive uses and of measuring the less tangible consequences of such allocation decisions. There is discussion of the very difficult subject of estimating the amenity value of land.

There are useful chapters on the statutory British planning system and on population trends and transport in

rural areas. A better understanding of all these matters would enable judgments to be made which used resources more efficiently, not only in cash terms, for society.

It can certainly be justly claimed that this is a useful source book for planners, landscape designers, students of environmental studies, agricultural economics and rural geography. There is an excellent list of references and a good index.

GEOFFREY BERRY

Free radicals

Free-radical Chemistry: Structure and Mechanism. D. C. Nonhebel and J. C. Walton. Pp. xv+572. (Cambridge University: London, January 1974.) £15; \$35.

RAPIDLY growing scientific subjects periodically need exposition in comprehensive textbooks and it is many years since this subject has received an adequate broad survey that meets the requirements of both senior undergraduates and research workers. Recent quantitative measurements, mainly by gas chromatography and ESR spectroscopy, have so greatly enhanced knowledge of chemical reactions that publications of only 10 years ago are no longer factually correct and contain some theoretical deductions that are no longer fully valid.

This book from Scotland, which is now a very active region for research in free radical chemistry, meets a real need. It is a collaboration between a physical (Dr Walton) and an organic chemist (Dr Nonhebel) who have surveyed harmoniously physical measurements, gas reactions, reactions of atoms and simple alkyl radicals, as well as a wide range of more complex reactions in solution. They have paid particular attention to recent work and theories.

Polymer chemistry has been omitted for lack of space. But though polymerisation technology is still expanding its theoretical basis is changing but little and has been covered reasonably well in a long chapter on reactions of alkyl radicals.

A particularly long chapter is accorded to radical oxidations and reductions. This, a rapidly expanding sector of free radical chemistry, certainly merits detailed treatment, but the text gives far too much space to phenol oxidation in comparison with autoxidation or possibly to reactions involving transition metal ions which, for students, deserved particular attention since they bridge the deleterious scholastic gap between inorganic and organic chemistry.

Only the price should detract from the wide use of this excellently produced book.

W. A. WATERS

Life in the sea

Biological Oceanographic Processes. By Timothy R. Parsons and Masayuki Takahashi. Pp. x+186. (Pergamon: Oxford and New York, October 1973.) £4.

THIS reads like the summary of an excellent four- or five-volume treatise that, unfortunately, has never been written. The authors insist that it is no more than an introduction to biological oceanography. A reader with no previous knowledge of the subject could, indeed, start from first principles. By page 10 he would reach the mathematics of diversity indices; four or five pages later he would cope with the application of statistics to the interpretation of samples; by page 21 he would have polished off biogeography and started on time-series variability.

The authors maintain this pace throughout. No new term is introduced without an excellent definition. One cannot even quarrel with their one non-definition: of detritus, they say that "while the word lacks definition it should not be regarded as lacking in importance" and then, having paused, off they go again and deal with the physics and chemistry of detritus in great depth—in four pages.

The book deals, almost exclusively,

with near-surface pelagic populations, their environment and their ecological processes. Benthic, littoral and bathypelagic systems are excluded. Indeed, almost everything is excluded that cannot be expressed in quantitative terms—and preferably summarised as an equation.

Some sections are superficial but what else can one expect in a book of such breadth? There are chapters dealing with the distribution and chemical composition of plankton, nutrients and seawater, heterotrophic and autotrophic processes, plankton feeding and production, organic and inorganic cycles and the transfer of energy and materials in the food chain. The authors claim that it is not a literature review but nearly 700 well-chosen references, alone, justify the purchase price of the book.

The last chapter, "some practical problems in biological oceanography", is trivial and unnecessary. Otherwise, this is an excellent book for professional oceanographers, biologists or, indeed, as the authors put it, "scientists in other professions who may require some quantitative expressions of biological oceanographic phenomena".

R. S. GLOVER

obituary

O. M. B. Bulman

PROFESSOR OLIVER MEREDITH BOONE BULMAN, FRS, who died at the age of 71 on February 18 was generally recognised as the leading authority on the graptolites, the group of colonial organisms which came into being, flourished and died out in the Lower Palaeozoic.

Bulman was trained as a geologist at Imperial College where he remained to carry out research under the direction of W. W. Watts. Among fellow students investigating various aspects of the Palaeozoic rocks of Britain at that time were Stubblefield, Whittard, Mitchell, Howell and David Williams all of whom were to hold posts of distinction.

Bulman has recorded that he was first attracted to the graptolites by beautifully preserved specimens of *Dictyonema* in the Sineton shales, on which he and Stubblefield were working; and he took this as the subject for his first palaeontological investigations. From the first, he concentrated on well

preserved material which he prepared and figured with great skill. In this way he was able to establish details of the external form, and in many instances, of the internal structure. With this new information he was particularly successful in elucidating the early stages of the growth of graptolites. When he completed his tenure of a Senior Studentship of the Royal Commission for the Exhibition of 1851, it was said that only a man of exceptional talent could have produced the results he had by then achieved.

After holding demonstratorships in the Zoology and Geology Departments at Imperial College, Bulman was appointed in 1931 to a post in the Sedgwick Museum where he remained for the rest of his academic life. In 1945 he became Reader in Palaeontology and in 1955 was appointed Woodwardian Professor of Geology. In 1945 he was elected a Fellow of Sydney Sussex College. He was President of Section C

of the British Association in 1959 and received in succession the Lyell Fund and the Lyell Medal from the Geological Society of which he was President from 1962–64. He was connected with the *Geological Magazine* either as Editor or its advisor for 40 years.

Throughout his career the study of graptolites remained his principle scientific concern. He continued as he had begun with a series of meticulous accounts which covered graptolites not only from Britain, but from North America, Scandinavia and Asia. He acquired an immense knowledge of their worldwide distribution which he put to use in his discussions of their mode of life and of the steps by which the graptolites evolved. He expressed his conclusions cautiously leaving the reader in no doubt where uncertainty existed.

Whatever the future may hold Bulman's part in the elucidation of the graptolites is likely to remain as a landmark in studies of Palaeozoic life.

Announcements

Appointments

The following are the members of the government select committee on science and technology: **R. Brown, R. Carter, J. Cunningham, N. McFarlane, A. Fletcher, D. Ginsberg, F. Hooley, I. Ledbetter, I. Lloyd, A. Neave, A. Palmer, N. Tebbit, C. Tyelar and K. Warren.**

D. J. Lyons has been appointed a member of the **Science Research Council.**

E. J. Denton has been appointed **Director of the Plymouth Laboratory** and **Secretary of the Marine Biological Association of the United Kingdom.**

G. R. Bainbridge has been appointed to the **Chair of Energy Studies at the University of Newcastle upon Tyne.**

I. R. Smith has been appointed to a chair in the Department of Electronic and Electrical Engineering at **Loughborough University of Technology.**

R. N. Parkins has been appointed to

the **William Cochrane Chair of Metallurgy and Engineering Materials at the University of Newcastle upon Tyne.**

C. McGreavy has been appointed to a second chair in the Department of Chemical Engineering at the **University of Leeds.**

G. T. Stevenson has been appointed a professor of the **University of Southampton.**

D. K. Bailey has been appointed Professor of Geology at the **University of Reading.**

The following have been elected **Foreign Members of the Royal Society:** **R. Dulbecco, G. H. Hitchings, G. P. S. Occhialini and J-P Serre.**

The following have been elected **Members of the National Academy of Sciences:** **R. D. Alexander, E. Anders, R. C. Atkinson, K. F. Austen, W. O. Aydelotte, J. W. Backus, D. Baltimore, L. M. Beidler, F. R. Boyd, Jr, M. K. Brakke, E. Braunwald, W. R. Briggs, T. C. Bruice, S. J.**

Buchsbaum, O. L. Chapman, J. A. Clements, G. L. Closs, W. G. Cochran, C. C. Cockerham, L. J. Cronbach, H. W. Davenport, C. A. Finch, W. H. Flygare, H. L. Fraenkel-Conrat, R. Freedman, J. Furth, D. C. Gajdusek, E. P. Geiduschek, I. Giaever, M. Gibbs, R. R. Gilruth, H. H. Goldstine, L. A. Goodman, R. W. Gould, K. I. Greisen, J. Gross, R. C. L. Guillemin, C. F. Hockett, H. S. Houthakker, F. S. Hulse, L. Hurwicz, J. Hurwicz, J. D. Isaacs, A. Javan, E. V. Jensen, E. R. Kandel, B. Kok, N. M. Kroll, H. D. Lasswell, P. F. Lazarsfeld, W. Leontief, E. B. Leopold, A. P. Lerner, R. Levins, D. L. Lindsley, Jr, F. J. Low, C. R. Lynds, R. S. MacNeish, J. L. Margrave, C. F. Mosteller, G. D. Mostow, H. J. Müller-Eberhard, H. N. Munro, T. M. Newcomb, A. B. Novikoff, J. P. Ostriker, C. K. N. Patel, R. G. Pearson, W. G. Pfann, L. J. Postman, D. M. Prescott, C. L. Prosser, A. E. Puckett, S. Ratner, W. H. Riker, H. Ris, H. E. Robbins, A. Robinson, G. W. Salisbury, R. Schmid, T. W. Schultz, W. R. Sears,

I. I. Shapiro, R. G. Shulman, L. T. Silver, E. M. Stein, De-W. Stetten, Jr, P. Teitelbaum, H. M. Temin, B. L. Vallee, K. M. Watson, J. S. Waugh, G. W. Wetherill, B. Widom, J. Wolfowitz, and J. B. Wyngaarden.

Awards

The Wellcome Prize for 1974 has been presented to J. H. Wilkinson.

The National Academy of Sciences has presented the Henry Draper Medal to L. Spitzer, Jr; the United States Steel Foundation Award in Molecular Biology to D. Baltimore; the Gibbs Brothers Medal to P. Eisenberg; the Selman A. Waksman Award to R. Dulbecco; and the NAS Award in Environmental Quality to G. E. Hutchinson.

The 1974 Lenin Prizes for science and technology have been awarded to: L. V. Keldysh, Y. G. Abov, P. A. Krupchitskii, V. M. Lobashev, V. A. Nazarenko, A. V. Kirsanov, N. V. Belov, A. I. Opanrin, I. I. Mints, V. D. Timakov, G. Ya. Kagan, A. I. Nesterov, E. M. Tareev and A. I. Strukov.

Miscellaneous

The George Von Hevesy Prize will be awarded for papers on the subject of Nuclear Medicine at the First World Congress of Nuclear Medicine in Tokyo (Professor W. Horst, Clinic of Nuclear Medicine, University of Zurich, Rami-strasse 100, 8006 Zurich, Switzerland).

The Edna McConnell Clark Foundation of New York announces a programme of support for research projects on schistosomiasis (Dr Donald B. Hoffman, Jr, Vice President, The Edna McConnell Clark Foundation, 250 Park Avenue, Suite 904, New York, New York 10017).

Erratum

In the article "Additional location of metalliferous sediments in the Red Sea" by R. D. Bignell *et al.*, (*Nature* 248, 127; 1974), as the result of a printer's error, the following corrections should be made. Page 128, line 15 should read "possibility exists of further deposits at depth. Analysis of . . ." and line 29 should read "possibility that they formed as a result of the oxidation of . . .".

International Meetings

May 22, **Herbal Remedies in Europe** (Professor J. M. Rowson, Pro-Vice-Chancellor, University of Bradford, Bradford, UK).

Reports and publications

Great Britain

- Arthritis: a Vitamin Deficiency Disease. By E. C. Barton-Wright. Pp. 32. (London: United Trade Press, 1973.) £1.25. [42]
National Radiological Protection Board. Code of Practice for the Display of Sources of Ionizing Radiations at Exhibitions. Pp. 13. (London: HMSO, 1973.) 18 p net. [42]
Wildfowl 24. Edited by G. V. T. Matthews and M. A. Ogilvie. Pp. 176. (Oxford and London: Blackwell Scientific Publications, 1973. Published for the Wildfowl Trust, Slimbridge.) £2; 6.50. [42]
Scotland Today—Economic Developments. By W. S. Robertson. (The Betts Brown Memorial Lecture). Pp. 21. (Edinburgh: Heriot-Watt University, 1974.) 50p. [42]
Annual Report of the National Museum of Wales, 1972/1973. Pp. 158. (Cardiff: National Museum of Wales, 1973.) [42]
Mercury as an Environmental Pollutant—a Bibliography. By D. Taylor. Pp. 135+78. (Brixham, Devon: Imperial Chemical Industries, Ltd, 1973.) [42]
Natural Environment Research Council—National Institute of Oceanography. Collected Reprints Nos. 831–885. (Wormley, Godalming, Surrey: National Institute of Oceanography, 1972.) [42]
The Art of Observation. By Charles H. Gibbs-Smith. Pp. 24. (London: Bedford Square Press of the National Council of Social Services, 1973. Distributed by RPS, Victoria Hall, East Greenwich, London SE10 ORF.) 30p. [62]
The Wellcome Trust. Interim Report, 1972/1973. Pp. 11. (London: The Wellcome Trust, 52 Queen Anne Street, London W1, 1974.) [62]
The Clean Air Year Book for 1974. Pp. 120. (Brighton: National Society for Clean Air, 136 North Street, 1974.) £1.25. [62]
Freshwater Biological Association. Scientific Publication No. 18: A Key to the British Freshwater Cyclopoid and Calanoid Copepods. By J. P. Harding and W. A. Smith. Second edition. Pp. 56. (Ambleside, Westmorland: Freshwater Biological Association, 1974.) [72]

Other countries

- Stockholm International Peace Research Institute. Report of Activities 1972/1973. Pp. 27. (Stockholm: Stockholm International Peace Research Institute, 1974.) [12]
Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 73-5: Current Research in the Geological Sciences in Canada, 1972–1973. Compiled by Thomas E. Bolton. Pp. vi+193. \$2. Paper 73-42: Fossil Dinoflagellates—Index to Genera and Species. By J. K. Lentini and G. L. Williams. Pp. 176. \$2. Bulletin 225: Quaternary Stratigraphy of the Moose River Basin, Ontario. By R. G. Skinner. Pp. 77 \$3. (Ottawa: Information Canada, 1973.) [12]
United States Department of the Interior: Geological Survey. Professional Paper 818-A: Hydrothermal Alteration Association with Beryllium Deposits at Spor Mountain, Utah. By David A. Lindsey, Harold Ganow and Wayne Mountjoy. Pp. iii+20. (Washington, DC: Government Printing Office, 1974.) 75 cents. [42]
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